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Intraspecific variation among populations of *Goniozus nephantidis* Muesbeck (Hymenoptera: Bethylidae) based on RAPD and ITS-2 rDNA sequences

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ABSTRACT: Intra specific variation in populations of *Goniozus nephantidis* Muesbeck collected from the States of Andhra Pradesh, Karnataka and Kerala in India was inferred from Random amplified polymorphic DNA (RAPD) analysis and sequence variation in internal non coding transcribed spacer 2 (ITS-2) region of rDNA. RAPD analysis for genetic variation among the populations using 11 primers fragmented out to 72 polymorphic and 10 monomorphic bands. Jacquard's similarity coefficient to compare paired populations and population cluster based on similarity matrix grouped Kerala and Karnataka parasitoid populations as one cluster (86% similarity) and that from Andhra Pradesh as an out group (13% similarity). PCR of ITS-2 region for the three populations resulted in 850 bp product. The length of the ITS-2 partial sequence compared were 467 bp for Karnataka and Kerala and 466 bp for Andhra Pradesh populations. Partial sequences of ITS-2 region in Karnataka, Kerala and Andhra Pradesh populations revealed two GC repeats between 127 & 132 bp and between 462 & 467 bp. The total content for guanine and cytosine was 56%, which was higher than that for adenine and thymine (44%). Intra species variation among the Karnataka and Kerala populations was minimal. The PCR-RAPD profiles indicated some intra-specific polymorphism and confirmed occurrence of variability in the ITS-2 region. Phylogenetic studies on *G. nephantidis* are reported for the first time.

KEYWORDS: *Goniozus nephantidis*, genetic variation, ITS-2, population, RAPD

INTRODUCTION

Goniozus nephantidis Muesbeck is a gregarious larval ectoparasitoid of the coconut black headed caterpillar, *Opisina arenosella* Walker, a serious pest of coconut in

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many South Asian countries. Severe infestation of the pest results in leaves appearing scorched with drastic reduction in yield (Ramachandran *et al.*, 1979). In severe outbreaks thousands of palms are affected and all fronds except three or four youngest ones are killed, leading to by drastic reduction in production (Pillai and Bhat, 1986). The percent parasitism by *G. nephantidis* varied in different states, *viz.*, 3.7–47.6 in Kollam district of Kerala (Sathiamma *et al.*, 1996), 31 in Mahuva district of Gujarat (Kapadia and Mittal, 1993), 28 in Guntur district of Andhra Pradesh (Manjunath, 1985) and 48 from Bangalore district of Karnataka (Nadarajan and Channa Basavanna, 1980).

Differences in the populations of the parasitoid within the species may result in a population that differs biologically from other non-specific populations. Unfortunately, determination of the efficacy of an individual parasitoid species has been hampered by difficulty in identification because the species are closely related and morphologically similar (Overholt *et al.*, 1997). The success of classical biological control programs depends critically on the accurate identification of the natural enemies in both the initial phase where the natural enemies are chosen, and also during release and the subsequent evaluation phases (Delucchi *et al.*, 1976). Therefore characterization of the parasitoid populations based on molecular approaches is necessary to assess the divergence or the homology within, particularly when the populations are morphologically similar.

Molecular techniques have come in handy in resolving some of these issues in addition to classical taxonomy. The RAPD technique amplifies random fragments of genomic DNA by PCR using single primer of arbitrary nucleotide sequences and detects genetic polymorphisms. It provides a powerful tool for identification of strains and the estimation of genetic variability between isolates (Williams *et al.*, 1990).

The internal transcribed spacer 2 (ITS-2) is a phylogenetic marker and is a non coding and rapidly evolving region which has been successfully used as a molecular marker to discriminate among closely related insect species, subspecies or populations (Toda and Komazaki, 2002; Alvarez and Wendel, 2003). A remarkable feature of this sequence is its high divergence between species.

ITS-2 region have been frequently used to evaluate phylogenetic relationships among species and varieties of insects (Marinucci *et al.*, 1999) and to analyze the genetic variation among different local populations of a given species (Alvarez and Hoy, 2002).

The objective of the present study was to develop a PCR based assay of the ITS-2 region of rDNA and RAPD of genomic DNA for detecting and distinguishing different populations of *G. nephantidis* for the genetic variations and or relatedness. The documented literature reveals that no phylogenetic studies on this genus or any of its species have been reported.

MATERIALS AND METHODS

Populations of the parasitoid obtained from the parasitized host larvae of *O. arenosella* from coconut orchards from Andhra Pradesh, Karnataka and Kerala were maintained

in the laboratory. The parasitoid was reared at 26 ± 1 °C and 65% RH under laboratory conditions, on mature larvae of *Corcyra cephalonica* (Stainton) adopting standard methods.

DNA extraction

The adult parasitoids were frozen in liquid nitrogen and stored at −70 °C. Genomic DNA isolated from individual adults of each population was homogenized in a 1.5 µl tube with 100 µl of lysis buffer (200 mM Tris HCl; pH 8.0, 70 mM EDTA; 2M Sodium Chloride; 20 mM Sodium metabisulphite) till a clear homogenate was obtained. To this was added 35 µl of 5% N Sodium lauryl Sarcosine and then incubated at 60 °C for 2 h. The mixture was centrifuged at 15,000 rpm for 15 min and the supernatant was collected, 13.5 µl of 10 M Ammonium Acetate and 135 µl of Isopropanol was added and the tubes were left overnight at −20 °C. The tubes were then centrifuged at 4 °C for 15 min at 15,000 rpm. The supernatant was discarded and DNA pellet rinsed with 500 µl of 70% ethanol and dissolved in 30 µl of TE buffer. Genomic DNA isolated from individual wasps was stored at −20°C until use.

Random amplified polymorphic DNA

Forty five random decamer primers (Operon Technologies, USA) were tested on template DNA for three populations so as to identify those giving good and scorable amplification products. Eleven primers that revealed clear and polymorphic patterns were selected for RAPD analysis (Table 1). PCR was performed in an i Cycler (Biorad Laboratories). Each reaction mixture (25 µl) for PCR reaction consisted of 20 pmol random primer, 10x Taq assay buffer, 1U Taq Polymerase, $MgCl_2$ (Genei, Bangalore), 10 mM dNTP (Eppendorf) and 50 ng/µl of template DNA. PCR was carried out for 40 cycles at 94 °C for 30 sec, 36 °C for 30 sec and 72 °C for 80 sec with initial denaturation at 94 °C for 3 min. The 40th cycle was followed by an extended primer extension step at 72 °C for 4 min. The products were analyzed by electrophoresis on 1.8% Agarose gel with Ethidium Bromide staining.

Repeatable fragments amplified by each primer were scored from each population for cluster analysis. All the bands in the range of resolution were scored, except for very faint bands. The data were analyzed using Numerical Taxonomy and Multivariate Analysis System (NTSYS 2.01) package (Rohlf, 1997). Similarity matrix was computed for each individual population (Virk *et al.*, 1995). Jacquard's similarity coefficient values for pair wise comparison between populations were calculated and a similarity coefficient matrix was constructed. The resultant similarity matrix was used to generate a tree by UPGMA method in NTSYS Pc2 software package.

PCR amplification of the ITS-2 region of rDNA

PCR was carried out in 0.2 µl microcentrifuge tubes. Amplification of the ITS-2 region was done with forward primer 5'-TGTGAACTGCAGGACACATG-3' and the reverse primer 5'-GTCTTGCCTGCTCTGAG-3' (Stouthamer *et al.*, 1999). PCR

TABLE 1. Primers used for analysis of the genetic diversity of the different *G. nephantidis* populations using RAPD-PCR

Primer	Sequence 5'-3'	Maximum number of markers generated	Polymorphic markers	Monomorphic markers	Polymorphism (%)
CO7	GTCCCGACGA	6	4	2	66.6
C12	TGTCATCCCC	5	5	–	100
C14	TGCGTGCTTG	8	7	1	87.5
C15	GACGGATCAG	7	5	2	71.4
C16	CACACTCCAG	3	3	–	100
C18	TGAGTGGGTG	9	7	2	28.6
C19	GTTGCCAGCC	6	5	1	83.3
KO1	CATTCGAGCC	7	5	2	71.4
KO2	GTCTCCGCAA	5	5	–	100
K15	CTCCTGCCAA	2	2	–	100
K19	CACAGGCGGA	4	4	–	100

was performed in 50 μl reaction volumes using 5 μl (10x) Taq assay buffer, 1 μl dNTP's, each in 10 mM concentration (Eppendorf), 1 μl forward and reverse primers (10 pmol/μl), 0.25 μl Taq polymerase (1U, Genei, Bangalore) and 50–100 ng of template DNA. The cycling conditions performed in an i Cycler (Bio-Rad Laboratories) were initial denaturation at 95 °C for 5 min, followed by 30 cycles of DNA denaturation at 94 °C for 1 min, primer annealing at 55 °C for 1min and primer extension at 72 °C for 2 min with final extension at 72 °C for 10 min. PCR products were electrophoresed on a 1.8% low melting point agarose gel (Genei, Bangalore). Gels were stained using ethidium bromide. 100 bp molecular weight maker (MBI Fermentas) was run along with the samples for reference.

Sequencing of PCR products

DNA fragments were extracted from the gel using the Qia Quick Gel Extraction Kit (Qiagen, Hilden, Germany). PCR products of the ITS-2 region were sequenced directly with the corresponding PCR primers on both strands. The sequencing of the ITS-2 region was determined in ABI Prism 310 DNA sequencer using Big Dye Terminator Method. The sequences for the ITS-2 region for Karnataka, Kerala and Andhra Pradesh populations were submitted to GenBank with Accession numbers EU016231, EU719071 and EU719072.

Phylogenetic analysis

Bioedit Version 7.0.4.1 program (Hall, 1999) was run to edit the sequences. The DNA sequences were aligned for the phylogenetic analyses using the CLUSTAL W computer programme. The evolutionary distances were computed by Kimura's two

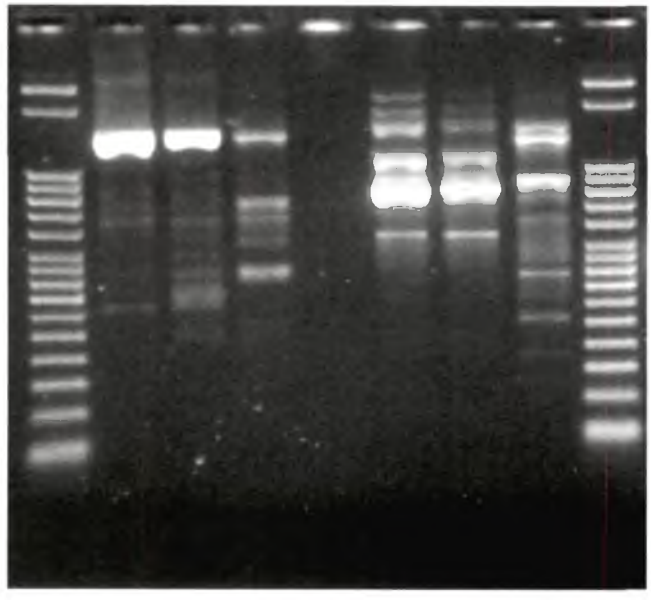

FIGURE 1. RAPD profile of *G. nephantidis* populations using 11 random primers.
In each gel, three primers for each of the three populations are shown, along with a size standard. (A) CO7, C12, C14, (B) C15, C16, C18, (C) C19, KO1, KO2 (D) K15, K16. (Lanes: M, 100bp DNA ladder); 1, 4 & 7 - Karnataka; 2, 5 & 8 - Kerala; 3, 6 & 9 - Andhra Pradesh.

parameter method (Kimura, 1980). The phylogenetic tree was constructed by the UPGMA method using MEGA version 3.1 (Kumar *et al.*, 2004).

RESULTS AND DISCUSSION

RAPD of genomic DNA of *G. nephantidis* populations

The genetic diversity among three populations of *G. nephantidis* from Andhra Pradesh, Karnataka and Kerala and was studied by RAPD analysis. Eleven primers evaluated, generated a total of 82 bands out of which 72 bands were polymorphic and 10 were monomorphic. The extent of polymorphism varied with each primer ranging from 71.43 to 100 percent. The highest number of polymorphic markers was produced by C19 (9) & C18 primers (8) and the lowest by K15 (3). The primers KO2, K15 and K19 produced 100 percent polymorphism in all the populations analyzed (Table 1). The high degree of polymorphism can be attributed to the nature of the genetic material and the different climatic conditions from where the populations were obtained. The parasitoid collected from Andhra Pradesh produced banding patterns distinctly different from those produced from Karnataka and Kerala. All the eleven primers produced unique banding patterns that could differentiate the geographic populations (Fig. 1). These markers can be efficiently used for the study of genetic

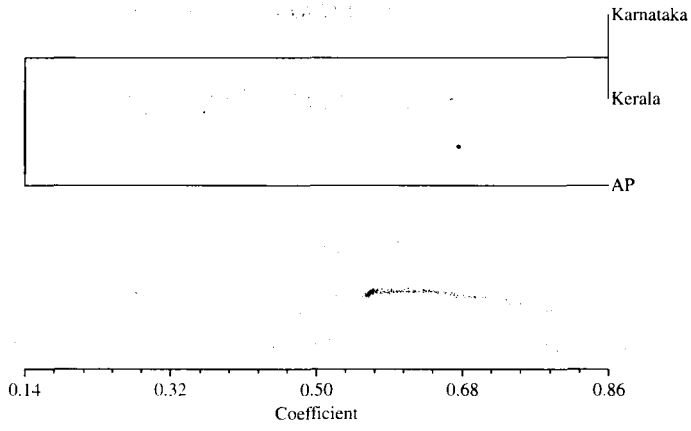

FIGURE 2. UPGMA cluster analysis–based dendrogram depicting genetic relationships among *G. nephantidis* populations with Jaccard's similarity values on scale.

variability because they make possible the random amplification of many genome regions that can be compared simultaneously.

The polymorphism revealed by RAPD serves as a dominant Mendelian marker (Williams *et al.*, 1990). In the present study, RAPD polymorphisms were analyzed with a phenetic distance measure (Jacquard's coefficient) from which a dendrogram was constructed to depict the genetic relationship among the parasitoid populations (Fig. 2). Clustering analysis based on the dendrogram showed two distinct groups. Populations from Karnataka and Kerala were grouped proximate with a high similarity coefficient of 86 % while the population from Andhra Pradesh was grouped distinct from the others with a low similarity coefficient of only 13 %. The dendrogram based on the genetic distances among the populations demonstrated the genetic specificity of the populations of the same taxonomic group. None of the populations of *G. nephantidis* shared a similarity more than 86% indicating some level of genetic differences between the populations.

Overall, within a similarity coefficient range of 0.13 to 0.86, clustering was in agreement with the geographical proximity from where the populations were obtained, except Andhra Pradesh population, which was distinct. Geographically, the states of Karnataka and Kerala have fairly similar cropping systems and the weather conditions that had significantly influenced the ecology and evolution of the parasitoids. Factors influencing the variations in the populations were reported in some of the insect pests. Host induced genetic variation as observed in thrips and other polyphagous insect pests such as *Helicoverpa amigera* (Scott *et al.*, 2003; Fakrudin *et al.*, 2004) is rather unlikely to be a cause for such a variation as all the populations were obtained from a common host *O. arenosella*. However, variations in the genome level may be corroborated due to differences in the sequence of nucleotides insertion of host DNA as in the case of viral bodies (Shapiro *et al.*, 1984). Further, the nature of the

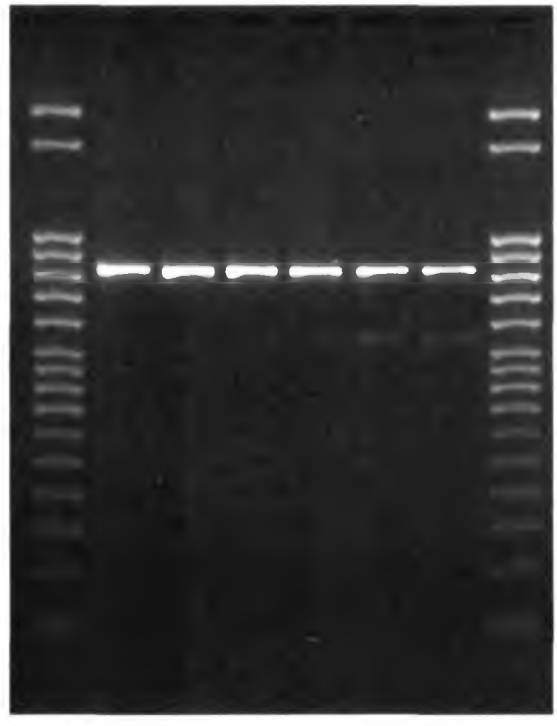

FIGURE 3. PCR amplified fragments of ITS2 obtained from different *G. nephantidis* populations. Lane 1, 2: *G. nephantidis*, Bangalore, Lane 3, 4: *G. nephantidis*, Kerala, Lane 5, 6: *G. nephantidis*, Andhra Pradesh.

habitat, topography of the location and the predisposing ecological factors governing the population dynamics of the parasitoid in the area from where they were obtained appear to have greatly influenced the variations and seem to have offset the other rationale for the differences observed in the present studies.

Variation in the ITS-2 sequences of the nuclear rDNA

Genomic DNA from the populations obtained from Karnataka, Kerala and Andhra Pradesh was amplified by PCR using ITS-2F and ITS-2R primers and produced a product of approximately 850 bp which was similar for all the three populations under study (Fig. 3). Sequences were confirmed as ITS-2 by Blastn similarity searching the Gen Bank database of National Center for Biotechnology Information (Altschul *et al.*, 1997). The length of the partial ITS-2 rDNA sequence was 467 bp for Karnataka and Kerala and 466 bp for Andhra Pradesh populations. DNA sequence variation in ITS-2 region among populations of *G. nephantidis* was compared. Variability consisted of single base changes or single insertions. There was 1.07% variation (a total 5bp at 5 sites) within 467 bp partial sequence between the Karnataka and Kerala populations

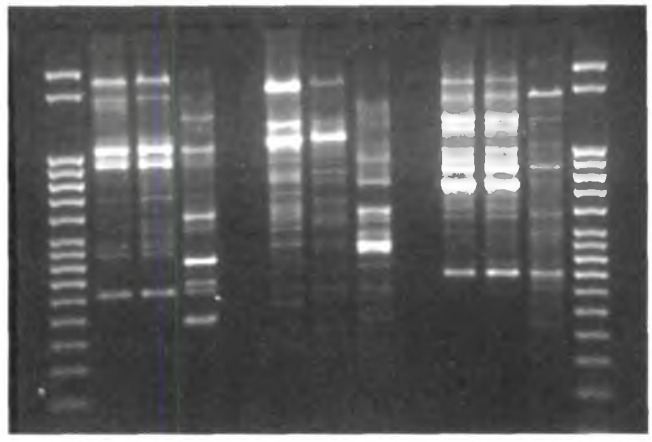

FIGURE 3. (A). Multiple alignments of ITS-2 partial sequences of Karnataka, Kerala and Andhra Pradesh populations of *G. nephantidis*. PCR amplified fragments of ITS-2 obtained from different *G. nephantidis* populations (Lanes: M, 100bp DNA ladder; 1 & 2 Karnataka; 3 & 4 Kerala; 5 & 6 Andhra Pradesh).

including single base pair substitutions. There was deletion of a nucleotide at 163 bp in the 466 bp sequence of Andhra Pradesh population causing the length variability. The simple sequence repeats observed among the three sequences, two GC repeats between 127 and 132 bp and between 462 and 467 bp on ITS-2 showed no variation in the repeats (Fig. 3A). Amplification of inter simple sequence repeat regions of *Microctonus aethiopoides* DNA demonstrated intraspecific genetic variation between French and Newzealand parasitoids (Phillips *et al.*, 2002). Differences in ITS-2 region between Karnataka and Kerala populations included 5 transversions (A/T, G/C) while between Kerala and Andhra Pradesh, 2 transitions (G/T) and 15 transversions were recorded. Only one transversion (position 270 of the aligned ITS-2 sequence) between the sequences of *Diadegma semiclausum* populations was recorded. There was one transition and one transversion (position 117 and 469 of the aligned ITS-2 sequences) between the ITS-2 sequences of *D. mollipla* from Kenya, Uganda and Tanzania populations (Wagener *et al.*, 2006). Total content of guanine and cytosine was 56%, which was higher than that for adenine and thymine (44%).

Phylogenetic analysis was performed using the ITS-2. Comparative analysis of partial sequences of ITS-2 produced a phylogenetic tree. Based on ITS-2 sequence analysis, two clusters were revealed comprising of Karnataka and Kerala populations in one and Andhra Pradesh in another that corresponds to the groups previously identified by RAPD-PCR. Analysis of sequences of the ITS-2 fragments supported the phylogeny inferred from the RAPD dendrogram grouping Karnataka and Kerala populations separately from the Andhra Pradesh population.

ITS-2 might indeed be a suitable marker not only for species and family level classification but also for megasystematics. Vogler and DeSalle (1994) analyzed the

sequence variations of the ITS-1 region of rDNA of *Cicindela dorsalis* in coastal areas of North America, and found two main clades in the Gulf of Mexico and the Atlantic Ocean in its phylogenetic tree. Alvarez and Hoy (2002) clarified the length variation of ITS-2 between Taiwanese and Australian populations of *Ageniaspis citricola*. Therefore, comparative analyses of DNA sequences have been used as an effective means for tracing the dispersal pattern of insects as well as for analyzing the genetic variation among insect populations (Ozaki and Ohbayashi, 2001; Osawa *et al.*, 2004). RAPD data analysis along with ITS-2 partial sequences reveal the same clustering and suggest that these techniques are appropriate tools for studying genetic variability among different populations.

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Molecular characterization of *Hirsutella* isolates from the eriophyid mite, *Aceria guerreronis* infesting coconut palm, by RAPD analysis

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ABSTRACT: Seven isolates of the mite specific fungal pathogen, *Hirsutella* spp. with distinct cultural and morphological characters obtained from 12 locations of Thrissur district were characterized using RAPD markers. Twenty five decamer primers of OPE and OPAH series, screened in RAPD analysis and five primers with good amplification, OPE-12, OPAH-5, OPAH-9, OPAH-13 and OPAH-15 were used for DNA characterization. Cluster analysis of the RAPD data using NTSYS programme exhibited genetic similarity among *Hirsutella* isolates. Two major clusters could be seen at 50 per cent similarity - Marakkal-I in one cluster and remaining six isolates, Chirakkekodu-I, Madakkathara-I, Konnakuzhy-I, Vellanikkara-I, Kanjirampally-I and Kanimangalam-I in the other.

KEYWORDS: coconut eriophyid mite, *Hirsutella* spp., RAPD

INTRODUCTION

Coconut palms in South India are affected by eriophyid mite, *Aceria guerreronis* Keifer (Sathiamma *et al.*, 1998). It lives beneath the bract and is well protected from pesticide application. Hence biological agents gain importance in technologies to manage the pest. *Hirsutella thompsonii* Fisher, the only fungal pathogen that has so far been extensively used all over the world to manage mite havoc is present in India on the mites. Beevi *et al.* (1999) isolated *H. thompsonii* var. *synnematosa* from eriophyid mite from Thrissur district, Kerala. Reliable identification of native isolates of natural enemies is crucial in its utilization in biocontrol programmes. Seven isolates of *Hirsutella* spp., differing in cultural and morphological characters, were

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obtained from 12 locations of Thrissur district. Recent developments in molecular biology have made characterization of DNA polymorphism using random primers possible (Williams *et al.*, 1990). Hence the DNA polymorphism of the above isolates of entomopathogenic fungi, *Hirsutella* spp. was studied.

MATERIALS AND METHODS

Oven dried fungal cultures of seven isolates of *Hirsutella* spp. differing in cultural and morphological characters were subjected to DNA characterization. The isolates of *Hirsutella*, two varieties of *H. thompsonii*, viz., *H. thompsonii* var. *thompsonii*, *H. thompsonii* var. *synnematosa* and *H. kirchnerii*, were studied in the laboratory. The DNA of the fungal cultures was isolated by following the protocol of (Murray and Thompson, 1980). The quality of DNA isolated was analysed on 0.9 per cent agarose gels. Then the gel was documented using Alpha Imager 1200 (Alpha Innotech Corporation, USA).

Primers under different Operon series viz., OPE (10 Nos.) and OPAH (15 Nos.) were screened for amplification of genomic DNA (extracted from the *Hirsutella* isolate Madakkathara-I) using the thermal cycle. From these, five primers which gave good amplification and performance were selected and utilized for further characterisation of the seven *Hirsutella* isolates.

The isolated DNA was subjected to RAPD analysis. The procedure of Williams *et al.* (1990) was modified and used for the amplification of fungal DNA. One cycle included (a) initial denaturation of 1 min at 94 °C, (b) samples were subjected to 35 cycles of denaturation (94 °C, 1 min), (c) primer annealing (35 °C, 2 min), (d) primer extension (72 °C, 2 min) and (e) final extension of 6 min at 72 °C. The reaction mixture (25 μl) consisted of (1) 10x Assay buffer with $MgCl_2$ - 2.5 μl, (2) dNTP mix 10 mM, (3) Primer 25 pM, (4) Taq DNA polymerase 1 μl (0.3 units), (5) Template DNA 25 ng/μl and (6) Sterile milli Q water to make up to 25 μl.

A master mix without primer and template was prepared using the reaction mixture for the required number of reactions. From this, 20.5 μl was pipetted into each PCR tube, and 1.5 μl of primer and 3 μl of template DNA were added. PCR tubes were loaded in the thermal cycles. The programme was run and it took 3 h and 45 min for completion. The amplified products were electrophoresed /size fractionated on 1.2 per cent agarose gel containing ethidium bromide using 1X TAE buffer. DNA fragments were viewed under UV light in transilluminator and then documented using Alphaimager.

The total number of bands alongwith the number of polymorphic bands obtained in different isolates with each of five primers used was recorded. The amplification profiles were compared and the bands of DNA fragments were scored as present (1) or absent (0) generating the 0, 1 matrices. The genetic similarity was estimated by computing DICE co-efficient using NTSYS PC-2.0 software programme (Dice, 1945; Nei and Li, 1979). The clustering was done and dendrograms were drawn by following unweighted pair group with metic mean (UPGMA) routine, in the above programme.

TABLE 1. Amplification bands obtained in screening of primers with *H. thompsonii* var. *thompsonii* isolate (Madakkathara-I)

Series	Sequence of primers	No. of amplification bands
OPE- 3	CCAGATGCAC	0
4	GTGACATGCC	0
5	TCAGGGAGGT	0
6	AAGACCCCTC	0
7	AGATGCAGCC	8
8	TCACCACGGT	0
9	CTTCACCCGA	0
10	CACCAGGTGA	0
11	GAGTCTCAGG	2
12	TTATCGCCCC	7
OPAH-1	TCCGCAACCA	4
2	CACTTCCGCT	5
3	GGTTACTGCC	7
4	CTCCCCAGAC	1
5	TTGCAGGCAG	5
6	GTAAGCCCCT	6
7	CCCTACGGAG	0
8	TTCCCGTGCC	5
9	AGAACCGAGG	7
10	GGGATGACCA	6
11	TCCGCTGAGA	1
12	TCCAACGGCT	3
13	TGAGTCCGCA	9
14	TGTGGCCGAA	2
15	CTACAGCGAC	6

RESULTS AND DISCUSSION

Of the seven isolates, the *Hirsutella* isolate, Madakkathara-I was subjected to DNA isolation for the primer screening. A discrete and intact DNA was obtained during the gel documentation. Out of the 25 decamer primers of OPE and OPAH series screened in RAPD analysis (Table 1), amplification was observed for 17 primers.

The following five primers were used for RAPD-PCR, OPE-12, OPAH-5, OPAH-9, OPAH-13 and OPAH-15. Based on the RAPD profile with the various primers, primer

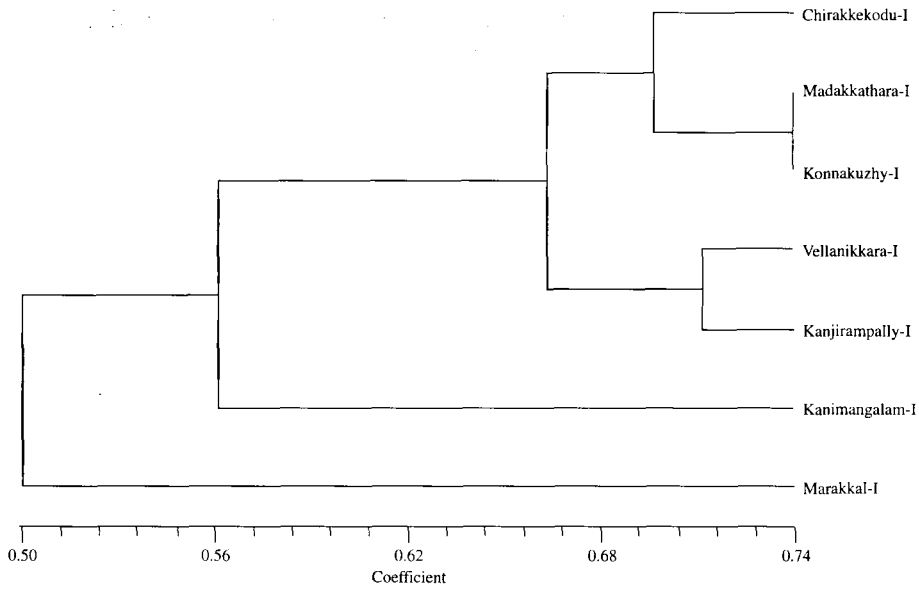

FIGURE 1. Dendrogram from the DNA analysis of seven *Hirsutella* isolates using five random primers

OPAH-13 was identified as the most promising one for characterizing the *Hirsutella* isolates. The numbers of amplifications obtained for seven isolates of *Hirsutella* with each of the five selected primers are shown in Table 2.

On the whole, the five selected operon primers generated a total of 57 bands with an average number of 11 bands per primer. The RAPD data were used to generate a similarity matrix using the SIMQUAL of the NTSYS programme. The phenetic representation of similarity co-efficients among seven *Hirsutella* isolates are presented in Fig. 1. In the dendrogram, the seven *Hirsutella* isolates were divided into two distinct major clusters at 50 per cent similarity. One cluster has only a single *Hirsutella* isolate, Marakkal-I. The first cluster with six isolates is again divided into three sub clusters. The first among this cluster 1A, has three isolates, Chirakkekodu-I, Madakkathara-I and Konnakuzhy-I, the latter two and cluster 1 having maximum similarity of 74 per cent. Vellanikkara-I and Kanjirampally-I formed the second major subcluster (1B) and a single isolate, Kanimangalam-I formed the third subcluster (1C). The isolates of cluster 1A and 1B together has 56 per cent similarity with cluster 1C while the isolates in cluster 1A and 1B has 67 per cent similarity.

H. thompsonii is polymorphic in several biological characteristics, including its potency to serve as a biocontrol agent. Molecular markers provide data that can assist scientists in choosing the most effective morph of the pathogen against a specific pest. In the present study also, molecular characterization showed genetic variation among the native isolates of the *H. thompsonii* and *H. kirchnerii*. Mozes *et al.* (1995) identified

TABLE 2. Amplification pattern of *Hirsutella* isolates using five selected random primers

Primer	Isolate						
	H1	H2	H3	H4	H5	H6	H7
OPE-12	1	2	2	1	4	0	1
OPAH-5	0	3	1	0	1	1	0
OPAH-9	4	5	1	5	3	4	0
OPAH-13	1	2	0	1	3	0	3
OPAH-15	3	1	1	0	1	1	1

H1 – *H. thompsonii* var. *thompsonii*; Chirakkekodu-I (*H.t.t.*Cdu)
H2 – *H. thompsonii* var. *thompsonii*; Marakkal-I (*H.t.t.*Mal)
H3 – *H. thompsonii* var. *synnematosa*; Vellanikkara-I (*H.t.s.*Vra)
H4 – *H. thompsonii* var. *synnematosa*; Madakkathara-I (*H.t.s.*Mra)
H5 – *H. thompsonii* var. *synnematosa*; Kanimangalam –I (*H.t.s.*Kam)
H6 – *H. thompsonii* var. *synnematosa*; Konnakuzhy-I (*H.t.s.*Khy)
H7 – *H. kirchnerii*; Kanjirampally-I (*H.k.*Kly)

two *Hirsutella* species (*H. necatrix* and *H. kirchnerii*) and six isolates of *H. thompsonii* by RAPD analysis. The occurrence of four numbers of same varieties of *H. thompsonii* var. *synnematosa* in different clusters of the dendrogram (1A, 1B and 1C) and those of *H. thompsonii* var. *thompsonii* in widely separated cluster 1A and 2 highlights the need for further studies on genetic similarities of morphology.

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Population dynamics of the root mealybugs, *Geococcus* spp. (Homoptera: Coccidae) infesting banana in Kerala

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ABSTRACT: The distribution of root mealybugs, *Geococcus* spp., around the corm of banana was assessed. Maximum population of 26 colonies/15 cm^3 soil sample occurred within 20-40 cm radius followed by 18.4 colonies at 40-60 cm and they were on par. In the case of vertical distribution, the highest population (23.2 colonies) was collected within 20 cm depth. The entire root mealybug population congregated within 40 cm depth from the soil surface and about 69 per cent, within 60 cm radius from the corm. The population flourished with the commencement of SouthWest monsoon in June and reached a peak in July, followed by a decline from September reaching the lowest level in January and remained low up to May. Out of six banana cultivars evaluated in an infested field, Nendran was the most susceptible with highest number of mealybug colonies (4.38), followed by Njalipoovan (2.55). Poovan and Robusta showed low levels of colonies ranging from 0.25 to 1.25 and Palayankodan and Kodappanillakunnan were completely free from infestation.

KEYWORDS: *Geococcus* spp., population dynamics, distribution in rhizosphere

INTRODUCTION

Root mealybug was reported as a serious pest posing threat to Nendran banana in Palakkad, Thrissur and Ernakulam districts of Kerala (Verghese *et al.*, 2000; Therattil, 2002). Later the insect was identified as*Geococcus citrinus* Kuwana (Smitha *et al.*, 2005). Occurrence of *Geococcus coffeae* Green along with *G. citrinus* was reported by Smitha (2007).

Adults and crawlers of the root mealybug suck sap from the lateral roots of banana and as a result, the roots turn brown at the site of colonization and the portion beyond the damage dries up in due course. Yellowing of lower leaves and burnt appearance of

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TABLE 1. Horizontal and vertical distribution of root mealy bug, *Geococcus* spp. in banana rhizosphere

Distance/Depth (cm) from the corm	Number of colonies/sample	
	Horizontal	Vertical
0–20	14.40[b]	23.20[a]
	(3.75)	(4.75)
20–40	26.00[a]	5.80[b]
	(5.10)	(2.45)
40–60	18.40[ab]	0.00[c]
	(4.32)	(0.71)
60–80	11.40[b]	0.00[c]
	(3.42)	(0.71)
80–100	15.06[b]	0.00[c]
	(3.89)	(0.71)

Values given are mean of 10 replications.
Figures in parentheses are square root transformed values. Means followed by the same alphabets do not differ significantly ($P = 0.01$).

leaf margins are the aerial symptoms on affected plants. The bunch size is reduced and filling of fingers is adversely affected (Smitha *et al.*, 2005). In severe cases, the plant topples down in wind. Considering the seriousness of this pest problem, distribution of the insect in the root zone and in different months of the cropping season, and relative susceptibility of the common varieties grown in the infested area were studied.

MATERIALS AND METHODS

The horizontal and vertical distribution of root mealybug in banana rhizosphere was assessed by taking soil samples ($15 \times 15 \times 15\ cm^3$) from infested gardens at distances of 20, 40, 60, 80 and 100 cm from the base of plant and from similar depths from soil surface after removing the top soil up to a depth of 10 cm. Samples were taken from the compact soil in blocks with a spade without disturbing the compactness. Four gardens were selected and ten banana plants were selected at random from each garden. The number of colonies in each soil sample was counted by splitting the soil block in layers. The lay out was treated as Completely Randomized Block Design and the data were subjected to ANOVA test and DMRT.

Cultivars of banana popular in infested locations of Kerala, *viz.*, Rasthali, Robusta, Njalipoovan, Kodappanillakunnn and Palayankodan were evaluated under field conditions to test their relative susceptibility. The experiment was laid out in farmer's field at Mannarkkad of Palakkad district in randomized block design with four replications for each variety. Four suckers were maintained for each variety per replication. The cultivars were grown in a heavily infested site in the area.

TABLE 2. Population of root mealybug at different locations during July 2005 to June 2006

Month (July 2005–June 2006)	No. of colonies/sample		Mean
	Thachampara	Karakurissi	
July	7.35[a]	8.73[a]	8.04[a]
	(2.80)	(3.04)	(2.92)
August	4.48[b]	4.28[b]	4.38[b]
	(2.23)	(2.19)	(2.21)
September	3.06[c]	2.48[c]	2.77[c]
	(1.89)	(1.73)	(1.81)
October	2.95[c]	2.80[c]	2.88[c]
	(1.86)	(1.82)	(1.84)
November	3.38[c]	3.00[c]	3.19[c]
	(1.97)	(1.87)	(1.92)
December	1.78[d]	2.05[cd]	1.91[d]
	1.57	(1.60)	(1.55)
January	1.45[d]	1.38[e]	1.41[e]
	(1.40)	(1.37)	(1.38)
February	1.65[d]	1.73[d]	1.69[de]
	(1.47)	(1.49)	(1.48)
March	1.93[d]	1.85[d]	1.89[d]
	(1.56)	(1.53)	(1.55)
April	1.65[d]	1.43[e]	1.54[e]
	(1.47)	(1.39)	(1.43)
May	1.53[d]	1.55[e]	1.60[de]
	(1.42)	(1.43)	(1.45)
June	4.50[b]	3.73[b]	4.11[b]
	(2.24)	(2.06)	(2.15)

Values given are mean of 10 replications. Figures in parentheses are square root transformed values. Means followed by the same alphabets do not differ significantly ($P = 0.01$).

RESULTS AND DISCUSSION

Horizontal and vertical distribution of the root mealybug in relation to the corm revealed significant variations (Table 1). Maximum population of 26 colonies/sample was observed in samples collected at 20-40 cm radius followed by 18.4 colonies/sample at 40-60 cm radius and they were on par. There was no significant difference in population in the remaining samples. About 69 per cent of the population was collected within 60 cm radius. In case of vertical distribution, the highest population (23.2 colonies) was observed in samples from the depth of 0-20 cm and it was significantly higher to samples from the remaining depths. It was followed by 20-40 cm depth (5.8 colonies). Entire population congregated within 40 cm depth from the soil surface. No mealybug was found at depths beyond 40 cms. This result agrees with the earlier report on the root mealybugs *Dysmicoccus brevipes*

TABLE 3. Relative susceptibility of common cultivars of banana to root mealybug in Palakkad district

Variety	Mealy bug colonies/15 cm^3 soil			
	February 2006	April 2006	June 2006	Mean
Njalipoovan	2.38^{d}	2.13^{d}	3.13^{c}	2.55^{B}
	(1.70)	(1.62)	(1.90)	(1.74)
Poovan	0.25^{g}	0.38^{fg}	0.63^{fg}	0.42^{D}
	(0.87)	(0.93)	(0.16)	(0.95)
Robusta	0.38^{fg}	1.25^{e}	1.38^{e}	1.00^{C}
	(0.93)	(1.32)	(1.37)	(1.21)
Nendran	4.50^{b}	3.38^{c}	5.25^{a}	4.38^{A}
	(2.24	(1.97)	(2.40)	(2.20)
Palayankodan	0.00^{h}	0.00^{h}	0.00^{h}	0.00^{E}
	(0.71)	(0.71)	(0.71	(0.71)
Kodappanillakunnan	0.00^{h}	0.00^{h}	0.00^{h}	0.00^{E}
	(0.71)	(0.71)	(0.71	(0.71)
Mean	1.88^{B}	1.79^{B}	2.60^{A}	
	(1.54)	(1.51)	(1.76)	

Values given are mean of 10 replications.
Figures in parentheses are square root transformed values. Means followed by the same alphabets do not differ significantly ($P = 0.01$).

(Cockerell) (Rajagopal *et al.*, 1982) and *Rhizoecus kondonis* Kuw. (Hung *et al.*, 1983; Godfrey and Pickel, 1998).

The highest population of root mealybug was recorded in July 2005 (7.35 and 8.73 colonies/sample in Thachampara and Karakurissi panchayats, respectively) (Table 2). This was followed by June population at both places (4.50 and 3.73 colonies), which were on par and also on par with August population (4.48 and 4.28 colonies). The population started declining from September 2005 and remained low up to May 2006.

It is obvious that the population increases with the commencement of SouthWest monsoon in June and reaches a significantly higher peak in July and starts declining with the cessation of rains in August and remains on par up to December. In January 2006, it reaches the lowest level and the level remains on par up to May 2006. Berlinger (1971) reported that grapevine mealybug population reached the peak between mid May and mid June, followed by a second smaller peak between October and November. But Ngeve (2003) reported that the impact of cassava mealybug, *Stictococcus vayssieri* Richard was severe in the dry season than in wet season.

Correlation analysis between root mealybug population and soil parameters showed a significant positive correlation between the population and soil moisture content (0.971) and rainfall (0.835). The correlation with maximum and minimum soil temperature was negative (-0.705 and -0.571 respectively) and significant.

The cultivars, Palayankodan and Kodappanillakunnan were completely devoid of root mealybug infestation (Table 3). The number of mealybug colonies was highest in

Nendran (4.38 colonies/sample) and it was significantly higher than the population in other cultivars. Njalipoovan also recorded relatively higher number of colonies (2.55 colonies/ sample) and it can be considered susceptible. Poovan and Robusta showing low levels of colonies ranging from 0.25 to 1.25/ sample may be treated as tolerant varieties.

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Effect of supplementing the feed with copper and zinc on the rearing performance of silkworm (*Bombyx mori* L.)

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ABSTRACT: Effect of supplementing copper and zinc in the silkworm feed on the economic traits of the silkworm, *Bombyx mori* L., was assessed. Copper sulphate administration at 5 to 100 ppm decreased the pupation rate from 81 to 52%, while zinc sulphate at 10 to 400 ppm decreased it from 63 to 57%. Combination of copper sulphate and zinc sulphate resulted in a decrease in pupation from 85 to 28%. Though this pupal mortality was significant in treated batches, effect on the actual cocoon yield was not significant except in the highest concentration tried (100 and 400 ppm of copper and zinc, respectively). Cocoon weights and shell percentages did not show statistically significant variations among the treatments. High concentration of copper sulphate used alone at 50 ppm and in combination with zinc (100 + 400 ppm) caused significant reduction in shell weight and shell percentage. The deleterious effect on survival was alleviated when 5% lime solution was added in the treatments before feeding the silkworms with treated leaves. The results indicate the desirability of treating mulberry leaves with lime when copper and zinc contents in mulberry leaves cross the safe limits.

KEYWORDS: silkworm, Copper, Zinc, intake levels, cocoon yield

INTRODUCTION

Physiological studies have established the importance of copper and zinc in growth and development of silkworms. Therefore, a few workers have attempted supplementation of mineral salt solutions on mulberry leaves to improve the economic characters of silkworms (Magadum *et al.*, 1992; Balamani *et al.*, 1995; Hugar *et al.*, 1998). Reports of favourable effect on silkworms which are fed with mulberry leaves after foliar application of micronutrients are also available in literature (Bose and Majumder,

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1996; Singh, 1997). It is observed that low doses of copper and zinc have beneficial effect on the growth and development of silkworms and cocoon character (Magadum *et al.*, 1992; Hugar *et al.*, 1998). But copper and zinc content in mulberry leaf fluctuates in relation to soil and associated conditions and can even accumulate in plants to reach hazardous levels to silkworms (Miyoshi *et al.*, 1978). Although these metals are essential, due to their propensity to accumulate in larval bodies, excess content in the feed is harmful. When mulberry gardens are irrigated with polluted water containing heavy metals, their content in soil and leaves are found to be higher than those of leaves raised with bore–well water (Chandrakala *et al.*, 2009). The exact concentrations of copper and zinc, singly or in combination (as they are present in mulberry leaves), at which they affect the growth and survival of silkworms adversely or enhance the economic characters needs to be precisely determined. The present study was conducted to evaluate the effect of applying zinc and copper sulphate solutions at varying concentrations on mulberry leaves fed to *B. mori*, on its rearing performance. The scope of using lime on the treated leaves as an ameliorant and consequent suppression of ill effects was also examined.

MATERIALS AND METHODS

Bombyx mori larvae (race NB4D2) were reared on mulberry leaves (*Morus indica* L. var. M5) from hatching till the third moult, following the recommended package of practices (Krishnaswami, 1986). Freshly moulted fourth instar larvae were used for the experiments. Copper sulphate, and zinc sulphate solutions were prepared in graded concentrations (Table 1) using distilled water. In normal mulberry leaves used for silkworm rearing copper content is around 1/3rd of zinc content and hence graded concentrations of copper sulphate used were in the range of 5 to 100 ppm and of zinc sulphate were within 10 to 400 ppm. They were evaluated separately and in combination. For assessing the effect of the treatments on development and cocoon production, the solutions were sprayed on mulberry leaves at 1 ml/10 g of leaves, dried for 10–15 min and fed to newly moulted fourth instar larvae (100 larvae in each lot). The treated leaves were given once daily after bed cleaning and subsequent feedings as per prescribed schedule were given with untreated leaves. Each treatment was replicated three times. At spinning stage, larvae in each replication were mounted on separate labelled mountages and cocoons were harvested on the sixth day. The number and weight of total cocoons (actual cocoon yield) as well as cocoon and shell weights were recorded and shell percentages were calculated. The cocoons were cut open and live pupae were considered for calculation of pupation rate. In the second experiment selected concentrations (Table 2) of copper sulphate and zinc sulphate prepared in distilled water were sprayed in two lots, one with 5% lime and another without lime.

Data were subjected to relevant statistical analysis (ANOVA or Student's t test) for assessing the significance of variations observed among the treatments.

TABLE 1. Effect of feeding mulberry leaves sprayed with copper and zinc sulphate solutions singly and in combination, on the rearing performance of *Bombyx mori*

Treatment	Actual cocoon yield (g)	Percent Pupation	Cocoon wt. (g)	Shell wt. (g)	Shell %
Water control	133.50	82.0	1.49	0.306	20.54
Copper sulphate 5 ppm	131.27	74.7*	1.49	0.296	19.87
Copper sulphate 10 ppm	123.27	72.0**	1.43	0.277	19.37
Copper sulphate 20 ppm	127.17	67.0**	1.46	0.290	19.86
Copper sulphate 50 ppm	118.85	59.0**	1.39	0.270*	19.42
Copper sulphate 100 ppm	119.55	52.3**	1.43	0.275	19.23
CD at 5%	NS	5.61	NS	0.022	NS
Zinc sulphate 10 ppm	122.25	63.0**	1.41	0.305	21.63
Zinc sulphate 50 ppm	121.39	63.7**	1.44	0.290	20.14
Zinc sulphate 100 ppm	126.29	54.3**	1.46	0.286	19.59
Zinc sulphate 200 ppm	126.10	54.7**	1.48	0.294	19.86
Zinc sulphate 400 ppm	121.72	57.3**	1.47	0.289	19.66
CD at 5%	NS	5.41	NS	NS	NS
Copper sulphate & Zinc sulphate 5 + 10 ppm	126.65	85	1.49	0.323	21.68
Copper sulphate & Zinc sulphate 10 + 20 ppm	114.40	78.3	1.43	0.287	20.07
Copper sulphate & Zinc sulphate 20 + 50 ppm	114.65	66.3*	1.36	0.280	20.59
Copper sulphate & Zinc sulphate 50 + 100 ppm	122.54	63.0*	1.48	0.294	19.86
Copper sulphate & Zinc sulphate 100 + 200 ppm	114.54	56.0**	1.43	0.287	20.07
Copper sulphate & Zinc sulphate 100 + 400 ppm	103.95*	28.0**	1.35	0.240**	17.78**
CD at 5%	18.9	10.59	NS	0.024	1.311

*Significant at 5% level; **Significant at 1% level; NS, Not significant when compared to control batch.

RESULTS AND DISCUSSION

The data are presented in Tables 1 and 2.

Copper sulphate at 5 to 100 ppm levels decreased the cocoon yield from 133 to 120 g but the variations in data were not significant. The pupation rate was reduced significantly, depending on the varying concentrations. However, no statistically significant decrease in cocoon weights could be observed in any of the treated batches

TABLE 2. Effect of treating mulberry leaves with copper sulphate and zinc sulphate with and without lime on rearing performance of *Bombyx mori*

Treatment	Percent pupation	
	Without lime	With 5% lime
Water control	**88**	92
Copper sulphate: 25ppm	**79**	86*
Copper sulphate: 50ppm	**62**	80*
Copper sulphate: 75ppm	**62**	77^{NS}
Zinc sulphate: 50ppm	**80**	84*
Zinc sulphate: 100ppm	**79**	83*
Zinc sulphate: 200ppm	**68**	82*
Copper sulphate & Zinc sulphate: 25 + 50 ppm	62	73*
Copper sulphate & Zinc sulphate: 50 + 100 ppm	55	73*
Copper sulphate & Zinc sulphate: 75 + 200 ppm	52	65*

*Significant at 5% level; ^{NS} Not significant.

though the lowest. i.e., 1.39 g was recorded at 50 ppm level as against 1.43 to 1.49 g in other batches. Though a decrease in shell weight was observed in treatments, significant reduction was noticed only at 50 ppm level. When zinc sulphate from 10 ppm to 400 ppm levels was administered, statistically significant decrease was noticed in percent pupation in different treatments (54.3 to 63%) compared to control (82%). No significant changes in either cocoon yield/weight or shell weights were evident in the data in different treatments including control.

The combination of copper and zinc sulphate at 5 + 10 ppm and 10 + 20 ppm levels of copper and zinc came on par with control with reference to pupation percentage while 20 + 50, 50 + 100 and 100 + 200 ppm level combinations were on par among themselves and deleterious. At the highest dose of 100 + 400 ppm percent pupation decreased to a very low level of 28%.

These results indicate that though zinc and copper are essential, excess content is harmful to the survival of silkworms. The earlier reports suggest that the influence of zinc or copper on silkworm depends on the concentration, either as supplements or in the feed itself. Magadum *et al.* (1992) observed that feeding the larvae with mulberry leaves soaked in low concentrations like 15, 30, 45 ppm solutions of copper sulphate significantly increased the economic characters of the silkworm and there was deleterious effect on survival at 50 and 100 ppm levels. In another report, zinc chloride at a lower concentration of 30 μg was found beneficial but deleterious at levels above 90 μg (Hugar *et al.*, 1998). Balamani *et al.* (1995) reported that zinc treatments up to 100 ppm level did not cause any adverse effect on nutritional indices or economic characters of silkworm. Adverse effects of heavy metal concentrations in feed have been reported extensively (Lokanath *et al.*, 1986; Hua *et al.*, 1998; Prince

et al., 2001). In the present study, deleterious effect was evident on the pupation percentages of *B. mori* larvae reared on mulberry leaves fortified with copper and zinc salts at doses ranging between 5–100 and 10–400 ppm, respectively. But significant variations were not observed in the treatments with respect to cocoon yield, cocoon weight, shell weight and shell percentage, when used alone or in combination. The lack of any favourable effect on silk production disagreeing with most of the earlier studies may be attributed to the higher content of these metals in mulberry leaves used for the experiment (copper upto 9 ppm and zinc upto 35 ppm). This bi-modal effect, i.e., stimulatory effect of zinc on metabolic activity at low levels and inhibitory at higher levels was reported by Hua *et al.* (1998). Insects regularly reared on such leaves might have developed some physiological adaptations to overcome the possible adverse effects of such heavy metals and that may be attributed as a possible cause for the lack response to higher doses of the metals used. These observations emphasise the need to take into consideration the content of such elements in the leaves used for experiments aiming at the assessment of dose limits for favourable and deleterious effects, the former for improving silk production and the latter for adopting preventive or ameliorating steps.

When the treatment of these metals was given in combination with 5% lime solution, the adverse effect on pupation rate was reduced as can be observed from the data presented in Table 2. The copper sulphate administration at 25 – 50 ppm was significantly better with lime treatment but at 75 ppm, the effect was not significant. In the case of zinc sulphate, significant effect of lime could be observed as the pupation percentage varied from 82–84% with lime treatment while in batches without lime treatment the range was 62–90%. When copper and zinc were used in combination, the pupation percentages ranged from 52 to 62% while with lime the same doses gave 65–73% pupation. So, when the mulberry leaves available for rearing have accumulated copper and zinc at high levels, the adverse effect can get reduced by the application of lime, as it is known that the absorption of metals is hindered under alkaline conditions. Hence, it can be concluded that close monitoring of heavy metal content in mulberry leaves is essential for the success of silkworm rearing.

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A survey of Euteliinae (Lepidoptera: Noctuidae) of Nilgiris, Tamil Nadu, India

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ABSTRACT: The Euteliinae fauna of Nilgiris is presented based on collection made during June 2006 to January 2009. Six species of Euteliinae under two genera are reported, with notes on their habits, habitats, economic importance and distribution. Among the six species, three species namely *Eutelia adulatricoides*, *Penicillaria jocosatrix* and *P. maculata* are reported for the first time from Nilgiris. A systematic account of all the six species incorporating genitalial morphology is given.

KEYWORDS: Lepidoptera, Noctuidae, Euteliinae, Nilgiris

INTRODUCTION

Moths belonging to the subfamily Euteliinae are generally more brightly coloured than members of other subfamilies of Noctuidae (Holloway, 1985). The forewings are either elongate or squarish. The subfamily Euteliinae is Oriental tropics and comprises more than 73 species (Holloway, 1985). Most of the Euteliinae species are defoliators and bore into young stems and shoots of mango and are economically important. The genus *Eutelia* can be easily recognized by the characters of thorax and stouter abdomen, the latter with a pair of anal tufts and extremely slight dorsal tuft on medial segments (Hampson, 1894). The genus comprises more than 24 species in Oriental region. Previously the pioneer worker, Hampson (1894) had reported 17 species in India, among which three species were in Nilgiris. There is no recent report on Noctuidae in Nilgiris Biosphere for more than four decades. Hence the taxonomy of Noctuidae needs to be updated.

During the expedition in Nilgiris, six species of Euteliinae under two genera were collected of which three species, namely *Eutelia adulatricoides*, *Penicillaria jocosatrix* and *P. maculata* are recorded for the first time from Nilgiris Biosphere.

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A systematic account of all the six species is given here since such a description incorporating genitalial morphology has not been published earlier.

MATERIALS AND METHODS

Moths of the subfamily Euteliinae were collected from Coonoor, Ooty, Doddabetta and Kothagiri in the Nilgiris Biosphere, during June 2006 to January 2009. The collection was made with a mercury vapour light trap (220/200w) source. Moths were also collected by sweep net. In each locality two random sites were selected for collection. The moths were identified with the help of dichotomous keys of Hampson (1894). The specimens are deposited at the Museum of Entomology Research Institute, Loyola College, Chennai 600 034.

RESULTS

Systematic account

Eutelia adulatricoides Mell, 1943

Material Examined: Tamilnadu (INDIA): Nilgiri District, Coonoor. 18. v. 2007, 7 ex.

Diagnosis: Wingspan 37 mm, forewing length 16 mm. Head black. Palpi, apex and frontal tuft speckled. Collar black, orange chestnut. Thorax black with orange paired tufts. Abdomen black dorsal tuft on medial segments. Forewing fuscous suffused with white, pale orange, traces with white subbasal line; large 'V' mark in the medial area, sinuous tripled postmedial line angled below the costa and excurved end of cell. Hind wing whitish, black in outer area. Underside black cell spot, indistinct medial line, thick band of postmedial line; outer margin pale yellow.

Male genitalia: Uncus short, broad and divided into three. Tegumen broad shoulder shaped. Juxta shield shaped. Vinculum short and broad, saccus V to U shaped. Valvae short, sacculus well developed, harpe blunt, cucullus well differentiated. Ampulla short, bearing hairs. Aedeagus broad with rod like cornuti; vesica with sickle shaped cornuti (Fig. 1a, b).

Female genitalia: Ovipositor lobes well developed, setose. Both pairs of apophyses well developed. Posterior apophysis long and thin; anterior apophysis short and broad with spatulate tip. Ductus bursae broad. Bursae copulatrix broad, membranous with parallel signum (Fig. 1c).

Distribution: Korea (North, South), Russia (RFE – Primorye migrant), Japan, China, Taiwan, India.

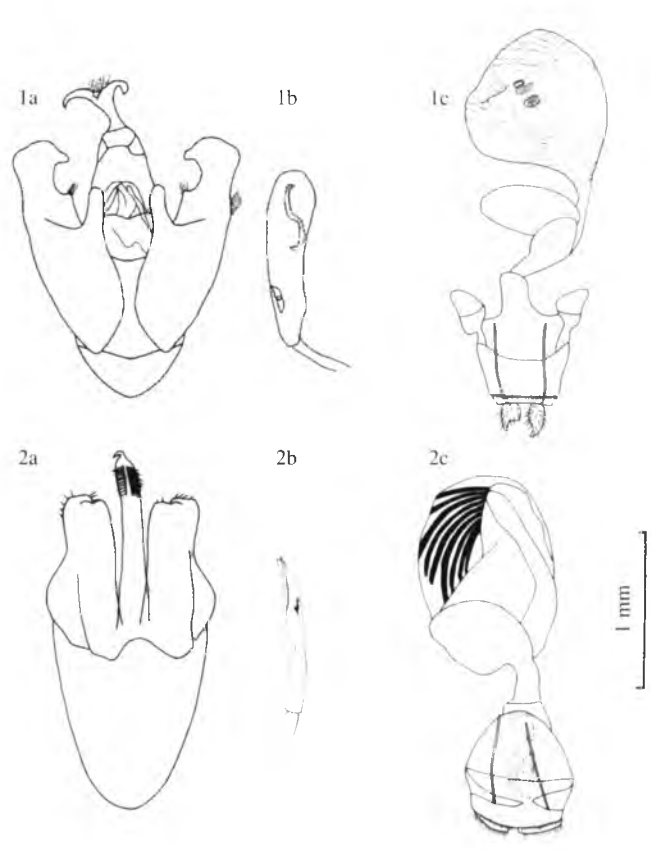

FIGURE 1. *Eutelia adulatricoides*: a, Male genitalia; b, Aedeagus; c, Female genitalia

FIGURE 2. *Eutelia delatrix*: a, Male genitalia; b, Aedeagus; c, Female genitalia

Eutelia delatrix Guenee, 1852

Material Examined: Tamilnadu (INDIA): Nilgiri District, Coonoor, 29. xi. 2006, 4 ex.; 1. i. 2007, 2 ex.; 8. xii. 2007, 1 ex.

Diagnosis: Wingspan 27-36 mm, forewing length 14-15 mm. Dull red brown. Labial palpi speckled. Forewing with basal half dark, bounded by a slightly oblique line with the outer area pale brown with a triangular chocolate patch before apex and three pale specks on it. The reniform narrow and grey with whitish edge, traces of numerous indistinct waved lines. Hind wing dark fuscous brown; the inner margin and vein 2 with some rufous specks; cilia rufous. Underside with basal and inner area paler.

Forewing grey towards apex and two postmedial lines; hind wing with black cell-spot and three minute wavy lines.

Male genitalia: Uncus well developed, long and slender apically hooked subapically setose. Tegumen weak. Valvae symmetrical. Vinculum long 'U' shaped; saccus broad. Aedeagus long and slender; vesica bearing 3 types of cornutus; apically well sclerotized cornuti, nail-like, triangular shaped cornuti (Fig. 2a, b).

Female genitalia: Ovipositor short, inferior strongly sclerotized. Posterior apophysis well developed, long and slender. Ductus bursae flattened short and stout. Bursae copulatrix rectangular with the presence of striated signum.(Fig. 2c).

Distribution: India, Sri Lanka, Burma, Java, Australia.

***Eutelia discistriga* Walker, 1865**

Material Examined: Tamilnadu (INDIA): Nilgiri District, Coonoor, 8. x. 2006. 1 ex. ; 19. v. 2007. 1 ex.

Diagnosis: Wingspan 28 mm, forewing length 12 mm. Palpi whitish, black at base, vertex of the head speckled. Forewing with brown basal region, indistinct antemedial line; pale medial band; three rufous wavy postmedial lines angled below the costa and with a large black 'V' mark on them beyond cell; an indistinct wavy submarginal line. Hind wing hyaline at base, outer area brown. Abdomen suffused with black and dotted laterally.

Male genitalia: Uncus short, broad and setose. Tegumen broad longer than vinculum. Valvae asymmetrical. Saccus broad and 'V' shaped. Aedeagus long and broad; vesica bearing rod like cornuti (Fig. 3a, b).

Female genitalia: Ovipositor lobes small, clothed with hairs. Posterior apophysis well developed, thin. Ductus bursae swollen. Corpus bursae membranous, spherical with pairs of signum (Fig. 3c).

Distribution: Aden, Karachi, Nilgiris.

***Eutelia jocosatrix* Guenee, 1852**

Material Examined: Tamilnadu (INDIA): Nilgiri District, Coonoor, 10. v. 2007, 3 ex.; 19. v. 2007, 1 ex.; 8. xii. 2007, 1 ex.

Diagnosis: Wingspan 24–26 mm ($n = 5$), forewing length 11 mm. Dark purplish red-brown. Forewing with traces of subbasal line; an indistinct antemedial line angled on median nervure; a postmedial line angled beyond cell with chocolate below the angle and joined by a chocolate patch from costa, inside indistinct submarginal, angled

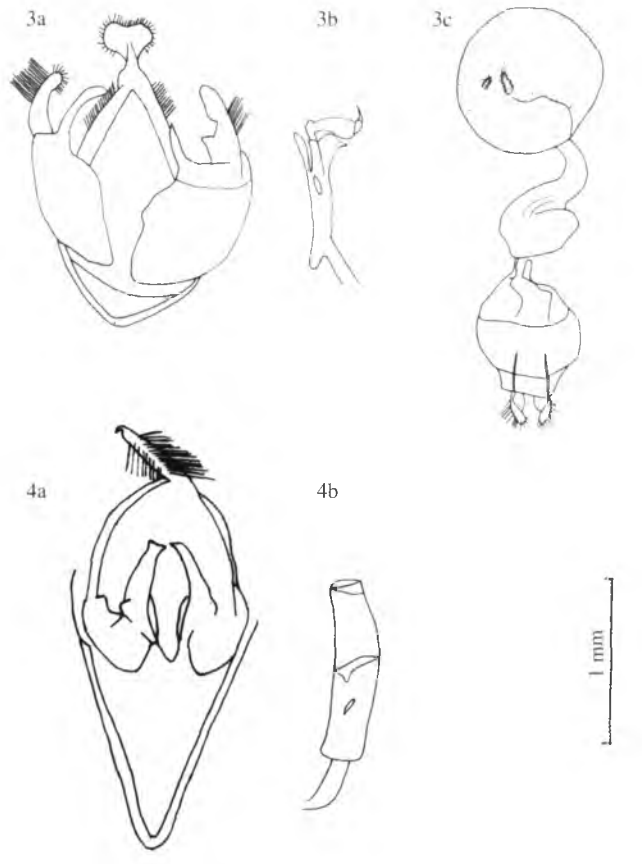

FIGURE 3. *Eutelia discistriga*: a, Male genitalia; b, Aedeagus; c, Female genitalia

FIGURE 4. *Eutelia jocosatrix*: a, Male genitalia; b, Aedeagus

whitish line. Hind wing short, the outer area purplish brown; underside with prominent cell-spot blackish.

Male genitalia: Uncus short, narrow, apically hooked and strongly setose. Tegumen broad, shorter than vinculum. Vinculum long 'V' shaped, saccus reduced. Valvae reduced well differentiated into costa and sacculus; sacculus broad and sclerotized. Juxta 'V' shaped sclerotized. Aedeagus short broad with rod-like cornuti. (Fig. 4a, b)

Female: Not studied.

Distribution: India, Sri Lanka, Java.

***Penicillaria jocosatrix* Holloway, 1985**

Material Examined: Tamilnadu (INDIA): Nilgiri District, Doddabedda, 29. ii. 2007, 1 ex.; Coonoor, 10. iv. 2007, 2 ex.; 7. vii. 2007, 1 ex.; 9. ix. 2007, 1 ex.; 22. x. 2007, 1 ex.

Diagnosis: Wingspan 22–28 mm ($n = 5$), forewing length 11-13mm. Forewing red brown; short subbasal line, antemedial line indistinct; a prominent curved medial line; reddish postmedial line broad; the submarginal subapically prominent as a fine white line. Hind wing basally white with a broad border of a slightly dull shade of the forewing colour. Underside with large brown cell-spot.

Male genitalia: Uncus short and stout covered setose. Tegumen broad and sclerotized. Vinculum longer than tegumen, 'U' shaped with extended narrow forming a prominent saccus. Valvae with bifurcated arms. Juxta small, triangular and sclerotized. Aedeagus broad, long; cornuti sword-like united with sickle shaped process (Fig. 5a, b).

Female genitalia: Ovipositor lobes well developed setose. Posterior apophysis well developed, long and thick. Ductus bursae short. Bursae copulatrix broad, membranous (Fig. 5c).

Distribution: Indo – Australian Tropics, Borneo.

***Penicillaria maculata* Butler, 1889**

Material Examined: Tamilnadu (INDIA); Nilgiri District, Coonoor, 22. x. 2007, 1 ex.

Diagnosis: Wingspan 25 mm, forewing length 10mm. Purplish red. Forewing traced with antemedial, medial lines; thick brown oblique postmedial line; submarginal is a subapical prominent white line; thick black fasciae on subtornal area. Hind wing basally white with broad border.

Male: Not studied.

Female genitalia: Ovipositor lobes prominent covered with long setae. Posterior apophysis well developed. Ductus bursae long, slender. Corpus bursae elongate without signum (Fig. 6a).

Distribution: Indo – Australian Tropics, Borneo.

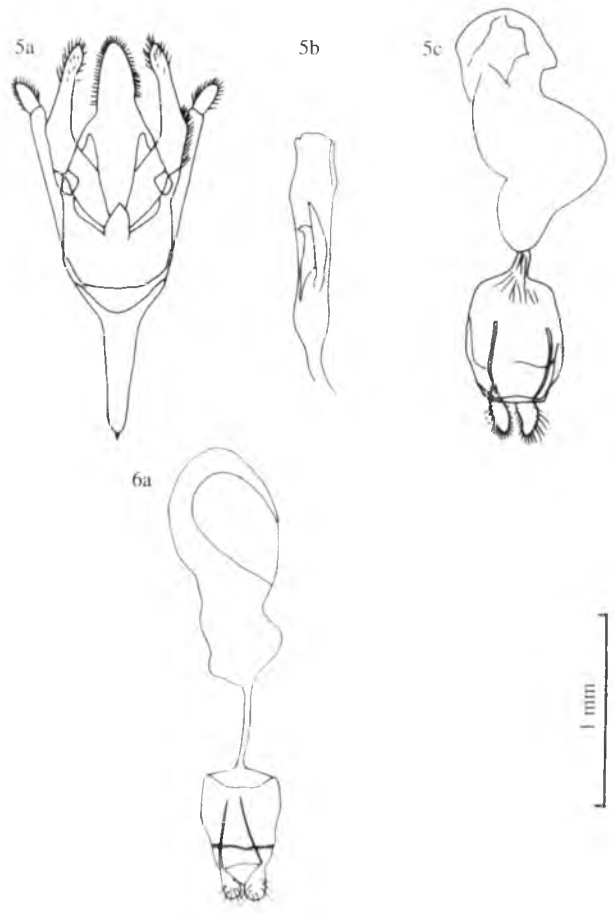

FIGURE 5. *Penicillaria jocosatrix*: a, Male genitalia; b, Aedeagus; c, Female genitalia

FIGURE 6. *Penicillaria maculata*: a, Female genitalia

DISCUSSION

The Euteliinae of Nilgiris is distributed throughout the study area, ranging in elevation from 1,858 to 2,623 m MSL. Among the six species collected, five species were documented from Coonoor locality and one species, *Penicillaria jocosatrix*, from higher elevation in Doddabetta only.

Four species belonging to the genus *Eutelia* were observed. The male genitalia of all the species of subfamily Euteliinae were studied. Uncus was long and narrow in *E. delatrix*. It was short and narrow, apically hooked in *E. jocosatrix* and divided into three in *E. adulatricoides*. Uncus was very short and broad in *E. discistriga*.

Vinculum was long and 'U' shaped in *E. delatrix*. Vinculum was longer than tegumen, 'V' shaped with extended narrow and forms a prominent saccus in *E. jocosatrix*. In *E. delatrix* the valvae was symmetrical, but reduced in *E. jocosatrix*. In the case of *E. discistriga* the valvae was asymmetrical. The ampulla was well developed in *E. adulatricoides*. Aedeagus was short, broad with rod-like cornuti in *E. jocosatrix* but long and narrow in *E. delatrix*. In *E. adulatricoides* the aedeagus had sickle shaped cornuti.

The female genitalia of six species belonging to the subfamily Euteliinae were studied. In all the species the posterior apophysis was strongly developed. In *E. adulatricoides* the ovipositor lobes were well developed. Anterior apophysis was short, broad with spatulate tip in *E. adulatricoides*. Bursae copulatrix was rectangular with presence of striated signum in *E. delatrix* whereas it was spherical in *E. discistriga*.

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A new species of *Hohorstiella* (Phthiraptera: Amblycera: Menoponidae) from Indian Ring Dove, *Streptopelia decaocto decaocto* (Columbiformes)

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ABSTRACT: A new species of the genus *Hohorstiella* (Phthiraptera: Amblycera: Menoponidae) collected from Indian Ring Dove, *Streptopelia decaocto decaocto* in district Rampur, U.P., India, is described and illustrated.

KEYWORDS: Phthiraptera, new species, *Hohorstiella*

INTRODUCTION

Genus *Hohorstiella* Eichler, 1940 resembles the genus *Menacanthus* (Carriker, 1949) but can be easily differentiated on account of the head spines, style of antennae, nature of pleurites and patches of setae. The generic characters of genus *Hohorstiella* have been listed by Rai (1977). As many as 21 species of *Hohorstiella* reportedly infest the Columbiformes birds (Price *et al.*, 2003). There are two groups in the genus *Hohorstiella,* the first group contains specimens in which pleurites are not prolonged while the members of second group have prolonged pleurites (Cicchino, 1978). Out of 21 species, 10 species belong to the first group and 11, to the second group (Carriker, 1949; Rai, 1977; Hill and Tuff, 1978; Tendeiro, 1980; Mey, 1984; Price and Emerson, 1986).

In the present study, specimens of the genus *Hohorstiella* collected from Indian Ring Dove, *Streptopelia decaocto decaocto* were found to be a new species.

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MATERIALS AND METHODS

Twenty four specimens (5 males, 12 females, 7 nymphs) were collected from two Indian Ring Doves (*S. decaocto decaocto*), during 2008, from district Rampur, U.P., India. Lice were macerated (10 % KOH), dehydrated (ethanol series), cleared (clove oil), mounted (Canada balsam), measured (ocular micrometer), and subjected to microscopy. Abbreviations used are TW, Temple Width; HL, Head Length; PW, Pro- thorax Width; ThL, Thorax Length; AL, Abdominal length; AW (IV), Abdominal Width of Segment IV; TL, Total Length.

HOHORSTIELLA RAMPURENSIS NEW SPECIES

Type material: **Holotype** one female and one male will be deposited in the Zoological Survey of India, Calcutta. These specimens were collected in district Rampur (located at 28°48′ N 79°00′E 28.8.1979) India, by Aftab Ahmad on 22.05.2008. **Paratypes** 4 males and 11 females, in the collection of Department of Zoology, Govt. Raza Postgraduate College, Rampur, U. P.

Diagnostic characters: The new species is characterized by the presence of 5 + 5 gular setae, nature of ocular slit, absence of median notch at the anterior tip of frons, straight margin of meta- thorax and the nature of male genitalia.

Type Host: *Streptopelia decaocto decaocto* (Frivaldszky)

Female (Figs. 1a and 2a–e)

Body colour pale brownish. Head broader than longer; frons convex in shape and bears two minute setae, anterior margin smooth. Pre- antennal region narrow, ocular slit straight and slightly narrow. Eyes well developed. Ocular fleck trilobed; ocular fringe depressed (composed posteriorly curved 13 setae; 2 long, 2 medium and 9 small), not projecting laterally. Temple narrow, slightly expanded, margin rounded. Setae 24 short, 25 longer in length. Alveoli of setae 26 and 27 closely associated; setae 26 short and fine while setae 27 long reaching up to posterior margin of the prothorax. Setae 28 and 29 long and 30, 31 short. Occipital margin straight with three long setae (21, 22 and 23 with alveoli in straight line) (Fig. 2d). Ventrum with well developed skeleton to support mandibles, reaching as far as on each side of clypeal region; peg like process arising near the base of palpi; clypeus narrow; mandibles weak; gular plate squarish, not well chitinized, bearing five setae on each side. Antennae four segmented, segment I short, antennule larger; segment III calciform, which is immediately inserted in the well marked depression of segment II; segment IV irregularly spherical. Maxillary palp four segmented. Post palpal process spinous. Comb brush with 17 short and fine setae. Gular plate with 5 + 5 setae (Fig. 2c).

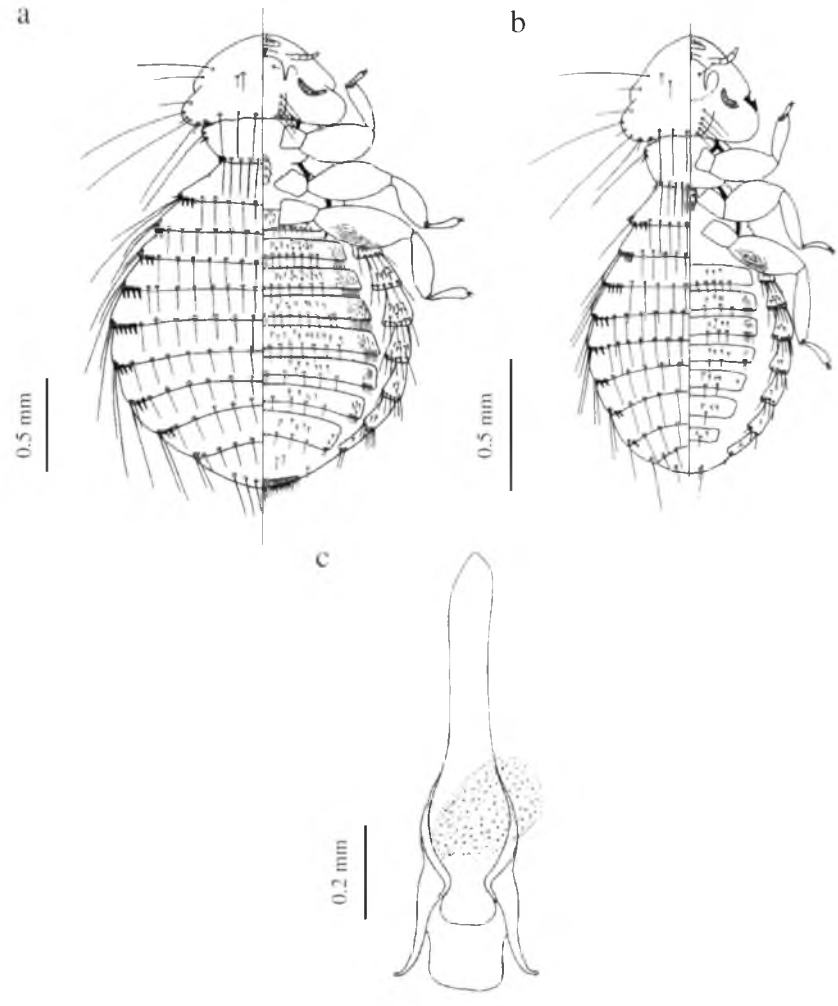

FIGURE 1. *Hohorstiella rampurensis*: a, dorso-ventral aspects of female; b, dorso- ventral aspects of male; c, male genitalia.

Pro-thorax large, expanded, lateral angles obtuse with one short spine and one long seta; posterior lateral margin straight; posterior margin with one short spine and 5 long setae on each half. Prosternal plate with 4+4 setae. Transverse bar and lateral bands well developed. Meso- thorax narrow, suture indistinct. Meta- thorax short, trapezoidal; posterior lateral margin slightly rounded with one spine and two long setae; posterior margin straight with three short spine and 5 long setae on each half. Legs well built, third femora with definite patch of 28 short setae. Inter coxal plate well pigmented.

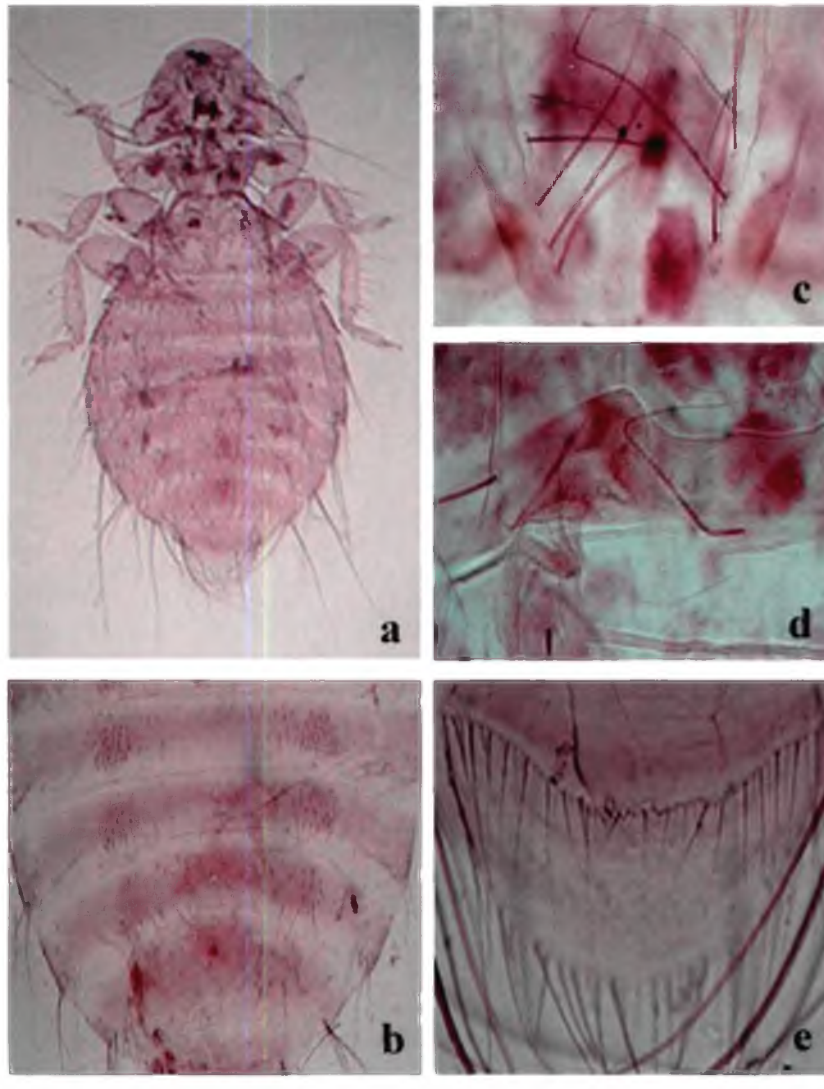

FIGURE 2. LM photograph of adult female *Hohorstiella rampurensis*: a, adult; b, posterior abdominal segments VI- VIII; c, gular plate; d, occipital setae 21,22 and 23; e, female terminalia.

Abdomen broadly rounded, thickest at IVth and Vth segment. Length of all segments almost equal, posterior margin of VIth, VIIth and VIIIth tergal segment concave (Fig. 2b). Sternal plates and pleural plates well marked. Elongation of ventral postero- medial angles of the pleurites absent. Posterior end pointed. Vulval margin

fringe with two rows of setae, upper row has 18 while, lower 20 (Fig. 2c). Abdominal chaetotaxy as follows:

Tergal setae: (n = 10), I 6–8 ($\bar{x}$ = 6.7), II 5-8 ($\bar{x}$ = 6.6), III 5–9 ($\bar{x}$ = 6.7), IV 5–8 ($\bar{x}$ = 6.4), V 5–8 ($\bar{x}$ = 6.3), VI 6–8 ($\bar{x}$ = 6.9), VII 4-5 ($\bar{x}$ = 4.8), VIII 3–5 ($\bar{x}$ = 4), IX 3 ($\bar{x}$ = 3). **Pleural Setae, Anterior setae:** II 4, III 5, IV 8, V 8, VI 3, VII 2, VIII 1 each side. **Marginal setae:** III 5, IV 5, V 6, VI 7, VII 6 and VIII 2 each side. **Sternal setae, Anterior setae:** I 4–6 ($\bar{x}$ = 4.6), II 11–15 ($\bar{x}$ = 12.4), III 12–14 ($\bar{x}$ = 12.6), IV 6–8 ($\bar{x}$ = 7.4), V 10–13 ($\bar{x}$ = 11.6), VI 5–8 ($\bar{x}$ = 7.2), VII 3–5 ($\bar{x}$ = 4), VIII 4–5 ($\bar{x}$ = 4.6) and IX 1–2 ($\bar{x}$ = 1.9) each side. **Marginal setae:** I 6–8 ($\bar{x}$ = 7.1), II 6–8 ($\bar{x}$ = 7.1), III 5–7 ($\bar{x}$ = 5.9), IV 7-10 ($\bar{x}$ = 8.7), V 5–8 ($\bar{x}$ = 6.9), VI 4–5 ($\bar{x}$ == 4.8), VII 3–5 ($\bar{x}$ == 4) and VIII 2–3 ($\bar{x}$ == 2.8) each side. **Sternal brushes, Anterior setae**: II 6–10 ($\bar{x}$ == 8.1), III 14-21 ($\bar{x}$ == 18.3), IV 14-21 ($\bar{x}$ == 17.8), V 8–13 ($\bar{x}$ == 10.9), VI 3–6 ($\bar{x}$ = 4.3), VII 3–5 ($\bar{x}$ = 3.3) and VIII 2–4 ($\bar{x}$ = 2.7) each side. **Marginal setae:** II 4–5 ($\bar{x}$ = 4.7), III 6–8 ($\bar{x}$ = 7.3), IV 8–9 ($\bar{x}$ = 8.7), V 4–7 ($\bar{x}$ = 5.7), VI 3–4 ($\bar{x}$ = 3.6), VII 3–5 ($\bar{x}$ = 4.1) and VIII 1–3 ($\bar{x}$ = 2.1) each side.

Measurements: (n = 3), TW = 0.62 (0.62–0.63), HL = 0.38 (0.38–0.39), PW = 0.44 (0.44–0.45), ThL = 0.43 (0.39–0.48), AW IV = 1.14 (1.07–1.19), AL = 1.26 (1.24–1.29), TL = 2.07 (2.01 – 2.11).

Male (Fig. 2b and 3a–f)

General morphology similar to female, smaller in length. Chaetotaxy more scarce. Frons slightly conical than female without median notch (Fig. 3d). Gular plate short and faintly sclerotized with 5+5 setae (Fig. 3e). Meta- thorax posterior margin straight with three short spine and 5 long setae on each half (Fig. 3b). Meta sternal plate with 3 + 3 setae. Elongation of ventral postero-medial angles of the pleurites absent (Fig. 3c). Femoral brushes have 23 short and fine setae. Terminal segment parabolic. Abdominal chaetotaxy as follows:

Tergal setae: (n = 4), I 4–6 ($\bar{x}$ = 5), II 4-5 ($\bar{x}$ = 4.7), III 6–7 ($\bar{x}$ = 6.2), IV 7–9 ($\bar{x}$ = 7.7), V 4–5 ($\bar{x}$ = 4.5), VI 2–4 ($\bar{x}$ = 3.2), VII 2-3 ($\bar{x}$ = 2.7), VIII 2 ($\bar{x}$ = 2). **Pleural Setae, Anterior setae:** II 3, III 5, IV 3, V 2, VI 2, VII 1 and VIII 1 each side. **Marginal setae:** II 5, III 6, IV 6, V 4, VI 4, VII 3 and VIII 1 each side. **Sternal setae, Anterior setae:** II 2–4 ($\bar{x}$ = 3), III 4 ($\bar{x}$ = 4), IV 4–6 ($\bar{x}$ = 5), V 3–4 ($\bar{x}$ = 3.7), VI 2–4 ($\bar{x}$ = 3.2), VII 3–4 ($\bar{x}$ = 3.2) and VIII 2 ($\bar{x}$ = 2) each side. **Marginal setae:** II 4–5 ($\bar{x}$ = 4.7), III 2–3 ($\bar{x}$ = 2.7), IV 2–4 ($\bar{x}$ = 3), V 1–2 ($\bar{x}$ = 1.7), VI 1–3 ($\bar{x}$ = 2), VII 1 ($\bar{x}$ = 1) and VIII 1 ($\bar{x}$ = 1) each side. **Sternal brushes, Anterior setae**: III 4-5 ($\bar{x}$ = 4.2), IV 3-5 ($\bar{x}$ = 4.2), V 2–3 ($\bar{x}$ = 2.2) and VI 1 ($\bar{x}$ = 1) each side. **Marginal setae:** III 4–6 ($\bar{x}$ = 4.7), IV 3–5($\bar{x}$ = 4) and V 2 ($\bar{x}$ = 2) each side.

Measurements: (n = 4), TW = 0.51 (0.51– 0.53), HL = 0.32 (0.32– 0.33), PW = 0.35 (0.33–0.37), ThL = 0.33 (0.31– 0.40), AW IV = 0.78 (0.77– 0.80), AL = 0.77 (0.70– 0.82), TL = 1.48 (1.42 – 1.59).

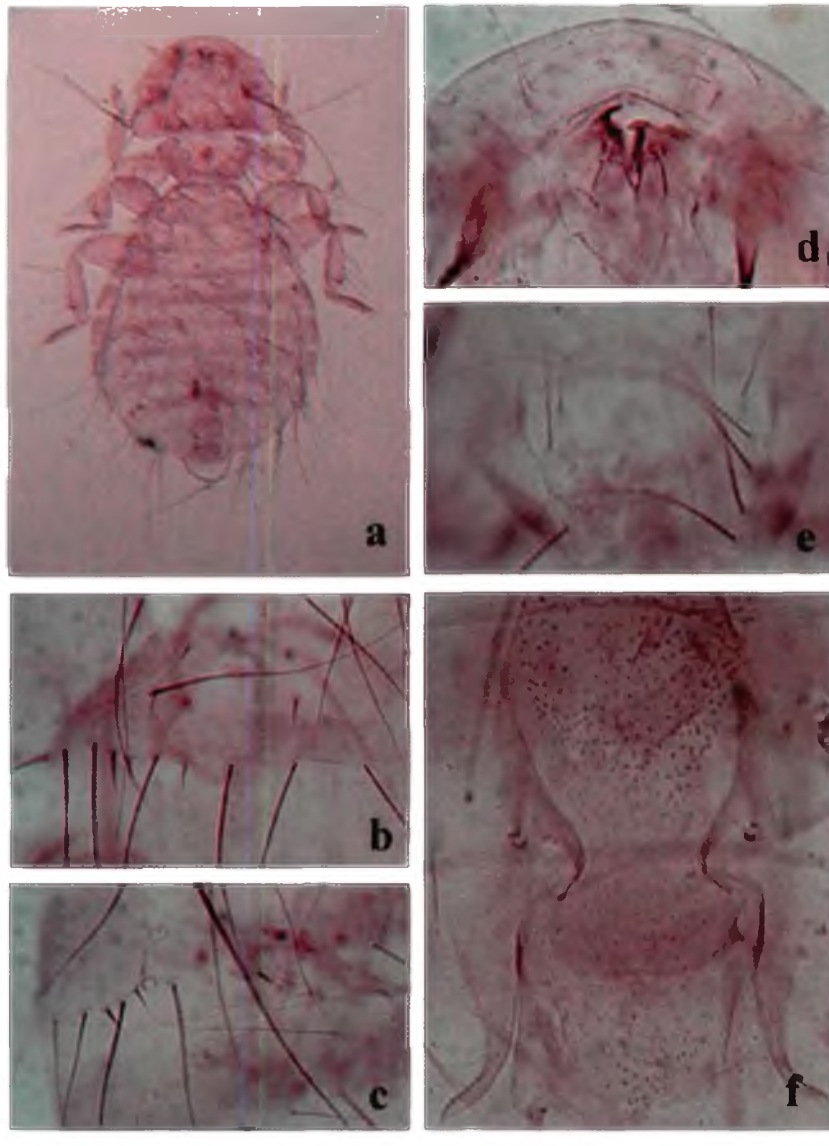

FIGURE 3. LM photograph of adult male *H. rampurensis*: a, adult; b, meta- thorax posterior margin; c, pleurite without elongation; d, frons anterior margin; e, gular plate; f, male genitalia.

Male genitalia

The basal apodeme of male genitalia is medium sized and conical at anterior end. Basal plate faintly chitinized. Parameres strong, thick anteriorly and curved outwards at posterior end. Endomeres comparatively thin, 'V' shaped curved at posterior end

and pointed at tip. Endomeral plate broad and rectangular. Nature of genital sclerite as shown in Fig. 1c and Fig. 3f.

DISCUSSION

Genus *Hohorstiella* is desperately under need of revision and complete characteristics of most of species described by authors have not been given. Even certain species have been described in absence of male specimens. Out of 10 species of *Hohorstiella* in which pleurites are not prolonged, three species reportedly occur on Genus *Streptopelia* (*H. asiatica* Fedorenko & Kekilova, 1978 occurs on *S. senegalensis*; *H. streptopeliae* Eichler, 1953 infests *S. turtur arenicola* and *H. modesta* Ansari, 1951 parasitizes *S. decaocto decaocto*).

The specimens of *H. rampurensis* differ from *H. modesta* in several characteristics i.e. absence of median notch at the anterior tip of frons; straight margins of metathorax (instead of concavo- convex margins); presence of occipital setae 21 (in addition to 22 and 23); 5 + 5 gular setae (instead of 4 + 4); 18 and 20 minute spines on upper and lower margins of vulva (in contrast to 12 and 13); concave posterior tergal margins of abdominal segments VI, VII and VIII (instead of straight margins) and the abdominal chaetotaxy.

The male genitalia of *H. rampurensis* n. sp. also differs from that of *H. modesta*. As far as other two species occurring on genus *Streptopelia* are concerned, *H. rampurensis* n. sp. differs from *H. streptopeliae* (infesting *S. turtur arenicola*) in the nature of ocular slit. It differs from *H. asiatica* (occurring on *S. senegalensis*) in having 5 + 5 gular setae, in contrast to 4 + 4.

Hence, the specimens collected from *S. decaocto decaocto* can be regarded as new species and named *H. rampurensis*.

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We are indebted to the Principal, Govt. Raza P.G. College, Rampur, U.P., for providing laboratory facilities, to Chief Wild Life Warden of U. P. for permission to catch the birds and to the UGC, New Delhi for providing financial support to A. K. S. in the form of Project No 38- 86/ 2009 (SR).

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Efficacy of some indigenous plant products for management of the aphid, *Aphis gossypii* Glover (Homoptera: Aphididae) infesting brinjal

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ABSTRACT: Two field experiments were conducted to test indigenous plant products viz. 5% extracts of Neem Seed Kernel (NSKE) and Chilli+Garlic, and 10% extracts of leaves of Neem, Parthenium, Lantana, Calotropis, Nerium, Tulsi and Marigold, including the chemical insecticide Chlorpyriphos (0.05%) against *Aphis gossypii* Glover infesting brinjal. The treatments reduced the populations of aphids significantly. NSKE with 51.92% and 54.07% reduction of population in the two experiments, followed by Chilli+Garlic with 47.11% and 48.77% reduction, and Calotropis with 45.24% and 46.38% reduction were on par, and they were also on par with Chlorpyriphos which gave 60.42% and 62.25% reduction.

KEYWORDS: aphid, *Aphis gossypii*, brinjal, botanicals

Brinjal is an important summer crop grown throughout India. The cotton aphid, *Aphis gossypii* Glover (Homoptera:Aphididae) is a major pest of the crop and it causes considerable damage to the foliage as well as reproductive parts. The nymphs and adults desap different plant parts causing deformities like curling, drying up and finally reduction in yield of the crop (Agarwala and Raychaudhari, 1981). In addition, it is capable of transmitting several viral diseases.

Plant products are gaining importance in crop protection, in view of their selective properties, low cost and safety to ecosystem. Studies were carried out to evaluate products from naturally occurring indigenous plants for their effectiveness against

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TABLE 1. Efficacy of different plant extracts sprayed against Brinjal aphid, *Aphis gossypii* in field

Treatment	Percent reduction of population	
	First spray	Second spray
Neem (*Azadirachta indica*) leaf extract, 10%	39.94 (39.17)	41.11(39.87)
Parthenium (*Parthenium hysterophorus*) leaf extract, 10%	29.76 (33.09)	30.12 (33.34)
Lantana (*Lantana camara*) leaf extract, 10%	32.50 (34.50)	33.75 (35.49)
Calotropis (*Calotropis procera*) leaf extract, 10%	45.24 (42.25)	46.38 (42.99)
Nerium (*Nerium oleander* L.) leaf extract, 10%	34.09 (35.73)	35.09 (36.33)
Tulsi (*Ocimum sanctum*) leaf extract, 10%	27.79 (31.82)	28.29 (32.14)
Marigold (*Tagetes erecta*) leaf extract, 10%	24.82 (29.87)	25.54 (30.33)
NSKE, 5%	51.92 (46.09)	54.07 (47.35)
Chilli+Garlic extract (*Capsicum annuum*+ *Allium sativum*), 5%	47.11 (43.34)	48.77 (44.51)
Chlorpyriphos, 0.05%	60.42 (51.00)	62.25 (52.06)
CD at 5% level	10.581	3.767

Values are mean of three replications and four observations. Figures in the parenthesis are arc-sine transformed values.

brinjal aphid. The experiment was carried out during 2005-06 at CORDET (Co-operative Rural Develoment Trust) IFFCO, Phulpur, Allahabad. The experiment was laid out in RBD with ten treatments (Table 1).

To prepare leaf extracts of Neem, Parthenium, Lantana, Calotropis, Nerium, Tulsi and Marigold, 100 g of chopped leaf was soaked in one litre of water for 12 h and 1% emulsifier was added to the filtrate. It was sprayed at the rate of 500 l/ha. NSKE was prepared by soaking 50 g of broken neem seed kernels in water overnight. A liquid detergent was added to the filtrate at the rate of 0.5ml /l. In case of Chilli+Garlic extract, 50 g each of fresh chilli fruit and garlic cloves were soaked in one litre of water each separately overnight and then ground into a paste and filtered through muslin cloth. Both the filtrates were mixed well and sprayed. The treatments were applied twice, at 120-125 days and 140-145 days of crop age. Aphid incidence was observed on five randomly selected plants per plot, taking six leaves, two each from upper, middle and lower strata. The observations were recorded one day before treatment as pre-treatment count and 1, 3, 7 and 11 days after spray as post-treatment counts. Per cent population reduction in different treatments over pre-treatment count was

calculated from the data using modified Abbot's formula (1925) as given by Fleming and Retnakaran (1985).

The results (Table 1) showed that NSKE, leaf extract of Calotropis and Chilli + Garlic extract were on par and they were as effective as the synthetic insecticide Chlorpyriphos. Similar results were obtained by Srinivasan and Babu (2001). The population reduction caused by botanicals might be due to their antifeedant/toxicant/hormonal effects. Similar findings were made by Satpathi and Ghatak (1990), Chitra *et al.* (1993) and Santhakumar *et al.* (2002).

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Description of female *Brueelia dicruri* Ansari 1955 (Phthiraptera: Ischnocera)

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ABSTRACT: Information is given on morphological features and chaetotaxy of female *Brueelia dicruri* (Ansari, 1955) as the female of this species has not been described earlier.

KEYWORDS: Phthiraptera, Mallophaga, Ischnocera, *Brueelia dicruri*

Survey of literature indicates that the female of *Brueelia dicruri* (Ansari, 1955) (Phthiraptera: Ischnocera) has not been described. A taxonomic description of male specimens of *B. dicruri* has been provided by Ansari (1955) but not that of the female. The present report provides a description of the morphological features and chaetotaxy of female *B. dicruri*.

Specimens of *B. dicruri* were collected from two white- bellied Drongos and permanent slides were made as suggested by Palma (1978). Abbreviations used are HW, Head Width; HL, Head Length; PW, Pro-thorax Width; PL, Pro-thorax Length; PtW, Ptero-thorax Width; PtL, Ptero-thorax Length; AW (IV), Abdominal Width of Segment IV; AL, Abdominal Length; TL, Total Length.

Brueelia dicruri

Female (Figs. 1, 2a–e): Head longer than wide, triangular, frons convex and shows slight concavity in the middle, one short seta present near it, two short setae on the ventrum below it. Dorsal anterior plate quardrate. Marginal carinae well formed but less sclerotized, ventral carinae uniformally sclerotized and continuous with the marginal carinae. Antenna five segmented, arises from traveulae (Fig. 2c), pedicel is larger than other segments, and all three flagellomeres are almost equal in size. Pre-antennal nodi well developed and continuous with the pre-ocular nodi. Eyes well developed, slightly protruded. Mandibular frame work well chitinized, oesophageal

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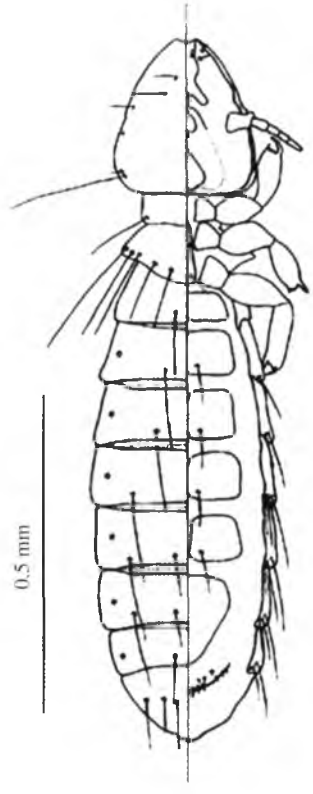

FIGURE 1. Dorsal and ventral aspects of female *Brueelia dicruri*

gland and lingual sclerite well developed. Ocular setae minute. Temple slightly rounded with one short and one long temporal seta (Fig. 2b). Occipital margin straight. Temporal carinae thin. Gular plate triangular in shape, bare.

Pro-thorax rectangular with one long seta at postero-lateral margin. Ptero-thorax trapezoidal, with convexo-concave posterior margin. Pteronotum with 5 very long posterior marginal setae, on each side. Meta sternal plate long with 2+2 setae (Fig. 2d).

Abdomen elliptical, tergal plates well developed, undivided. Sternal plates well pigmented and medially divided from IInd to VIth segment, VIIth and VIIIth sternal plates fused and form broad, heart shaped plate. Pleurites well developed, highly chitinized with re-entrant head (Fig. 2e). Terminal end bulbous. Vulvul plate 'V' shaped (Fig. 2a) with 10–12 minute spines.

Tergal setae: ($n = 2$) II, 1($\bar{x} = 1$); III, 1($\bar{x} = 1$); IV, 1($\bar{x} = 1$); V, 1-2($\bar{x} = 1.5$); VI, 1-2 ($\bar{x} = 1.5$); VII, 2($\bar{x} = 2$); VIII, 2($\bar{x} = 2$); IX, 2-3($\bar{x} = 2.5$) each side. **Sternal setae** III, 1($\bar{x} = 1$); IV, 1($\bar{x} = 1$); V, 1($\bar{x} = 1$); VI, 1-2($\bar{x} = 1.5$) each side. **Pleural setae** III, 1 + 1; IV, 2 + 2; V, 3 + 3; VI, 3 + 3; VII, 3 + 3; VIII, 2 + 2.

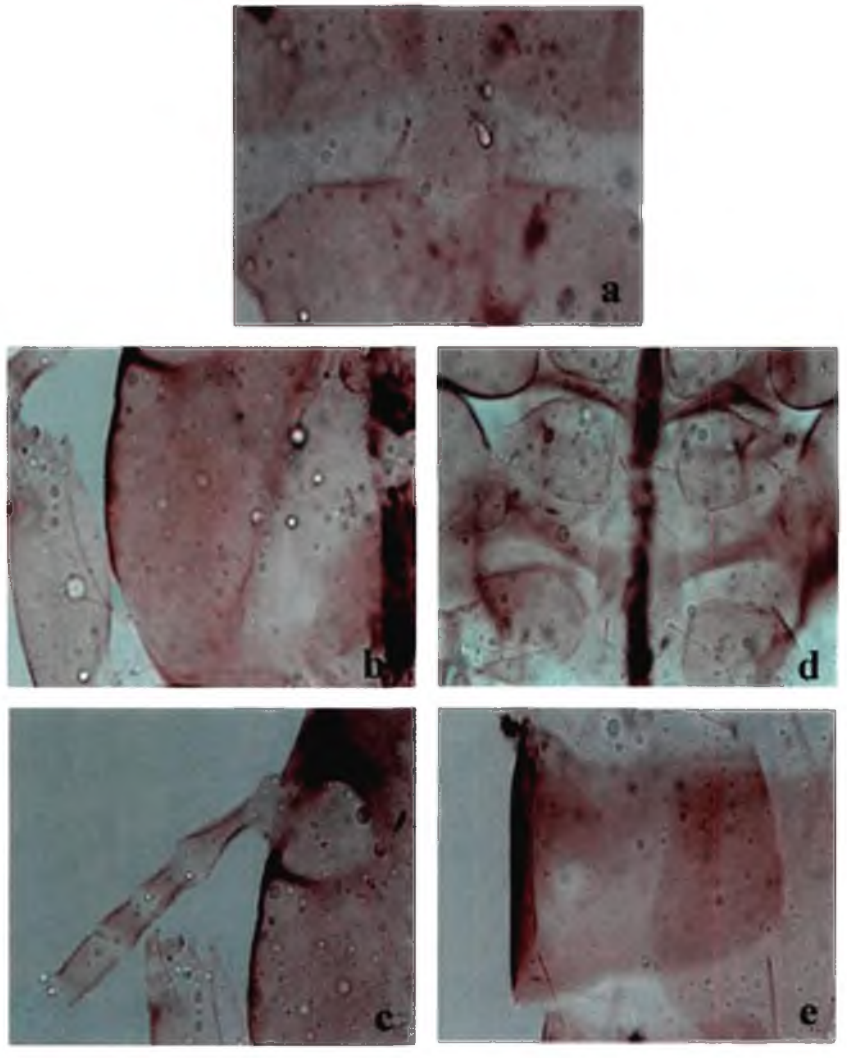

FIGURE 2. LM photographs of female *Brueelia dicruri*: (a) Enlarged view of vulval margin X 235, (b) Enlarged view of temple X 425, (c) Female antenna X 450, (d) Enlarged view of meta sternal plate X 475, (e) Enlarged view of pleural plate showing re-entrant head X 370

Measurements (mm): HW = 0.29, HL = 0.37, PW = 0.18, PL = 0.07, PtW = 0.29, PtL = 0.14, AW IV = 0.44, AL = 1.22, TL = 1.81.

Genus *Brueelia* contains over 280 described species and is one of the largest genera of lice in the suborder, Ischnocera (Price *et al.*, 2003). It occurs on at least 41 families of birds, in at least four orders. Since *Brueelia* is so speciose, no comprehensive revision of this genus has ever been performed. Hence, any comprehensive key for

the identification of species is not available. For the revisions and species descriptions in this group, the louse materials only from closely related hosts have been considered. For example, Ansari (1956, 1957) revised the species of *Brueelia* infesting the Corvidae (crows and jays, Passeriformes) and Dalgleish (1971) revised the species found on Picidae (woodpeckers, Piciformes).

Present report records information on the morphological features and chaetotaxy of female *B. dicruri*, not described so far.

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Nature of placodean sensilla of four ischnoceran Phthiraptera

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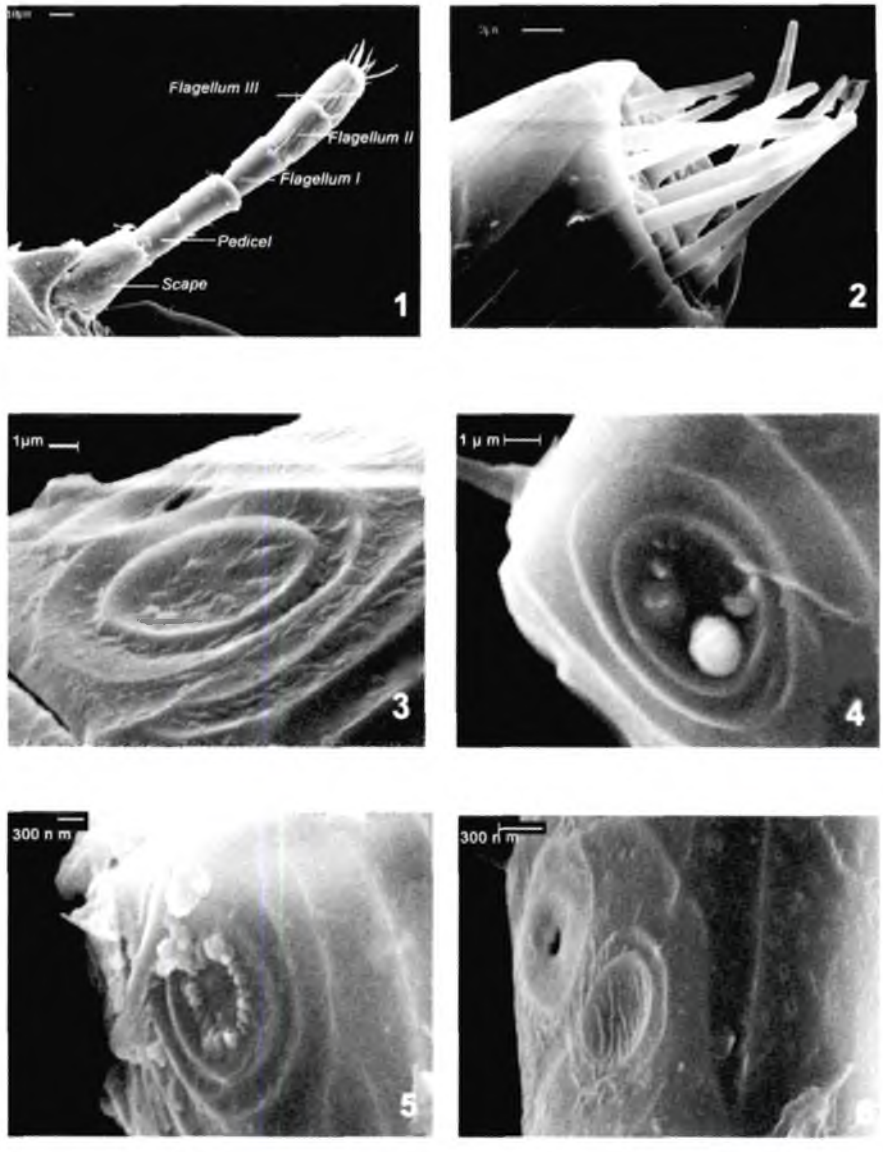
ABSTRACT: SEM study revealed that placodean sensilla occurring on the antennae (fourth and fifth segment) of four species of *Brueelia* species (infesting the red avadavat, mountain laughing thrush, common baya and house crow) exhibit diversity in nature.

KEYWORDS: Phthiraptera, Mallophaga, Ischnocera, *Brueelia*, antennal sensilla

Phthirapteran antennae are the principal sites of sensory structures. The antenna of ischnoceran Phthiraptera consists of three components viz. basal scape, pedicel and the flagellum. The flagellum is further divided into three segments. Selected workers have noted the nature of sensory equipment present on the antennae of certain avian lice (Neuffer, 1954; Clay, 1970; Slifer, 1976; Eichler *et al.*, 1976; Zlotorzycka and Kassner, 1986; Cicchino and Abrahamovich, 1988; Bhatnagar *et al.*, 2004). In the present paper an attempt was made to compare the nature of placodean sensilla of four species of *Brueelia* (*B. saliemi*, *B. plocea*, *B. sehri* and *B. amandavae*), in order to observe its taxonomic significance.

House crow (*Corvus splendens*) louse, *B. saliemi*, Himalyan laughing thrush (*Garrulax lineatus lineatus*) louse, *B. sehri*, baya (*Ploceus philippinus*) louse, *B. plocea* and red avadavat (*Amandava amandava*) louse, *B. amandavae* were collected from their respective hosts. For SEM studies the lice were stored in a mixture of 70% alcohol and 30% ether, cleaned (distilled water), dehydrated and mounted on double sided tape, gold coated and viewed under SEM.

Cicchino and Abrahamovich (1988) have reviewed the terminology adopted by previous workers to describe the sensorial equipment of phthirapteran antennae. All the segments of ischnoceran antennae bear varying numbers of tactile setae/position setae. Fourth and fifth antennal segments bear placodean sensilla and coelomic sensilla. The placodean sensilla of ischnoceran antennae generally appears as a saucer



FIGURES 1–6. 1. Antenna of *Brueelia amandavae*; 2. Third flagellomere of *B. saliemi*; 3. Placodean sensilla of the third flagellomere of *B. saliemi*; 4. Placodean sensilla of the third flagellomere of *B. plocea*; 5. Placodean sensilla of the third flagellomere of *B. sehri*; 6. Placodean sensilla of the third flagellomere of *B. amandavae*.

shaped structure with central raised area surrounded by varying number of radiating ridges separated by grooves. On the other hand, sensilla coeloconica occurs in the form of a distinct pore surrounded by cuticular ridges. The apex of fifth antennal segment bears 10-12 setae/pegs (Fig. 1, 2).

In *B. saliemi* a shallow circular groove occurs inside the saucer shaped placodean sensilla (Fig. 3). In case of *B. plocea*, four protuberances of varying sizes occur inside the saucer shaped area of placodean sensilla (Fig. 4). In *B. sehri* 16–17 granular protuberances appear to be arranged in a circular row inside the saucer (Fig. 5). In *B. amandavae* the placodean sensilla occurs in the form of a hollow saucer (devoid of any structure inside it) (Fig. 6).

Thus the present study suggests that the nature of placodean sensilla may differ species wise. The morphological features of placodean sensilla may have taxonomic significance. Cruz and Mateo (1996) examined the antennal sensilla of eight species of *Bovicola* (infesting mammals) and found that nature of pit organs occurring at the base of sensilla coeloconica (present on the third segment of antenna) exhibited diversity in shape in different species.

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Impact of intraspecific competition in the predation of the cotton bollworm, *Helicoverpa armigera* (Hübner) by *Scipinia horrida* (Stål) (Hemiptera: Reduviidae)

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ABSTRACT: The impact of intraspecific competition on predation by the assassin bug, *Scipinia horrida* (Stål) (Hemiptera: Reduviidae) on cotton bollworm, *Helicoverpa armigera* (Hübner) was investigated. Intraspecific competition increased predation by decreasing the latency to capture, paralyze and suck the prey. Competition also reduced the number of piercing sites on prey during feeding. These effects were proportionally increased with increase in competition. The impact of intraspecific competition was greater among the adults, which are solitary predators, compared to nymphal instars that feed in groups.

KEYWORDS: Assassin bug, *Scipinia horrida*, intraspecific competition, cotton bollworm, *Helicoverpa armigera*

Intraspecific competition among reduviid predators affects predatory efficiency and results in cannibalism (Ambrose, 1999). Intraspecific competition among nymphal instars of harpactorine reduviids viz., *Sinea diadema* (Taylor and Schmidt, 1994, 1996), *Irantha armipes* (Das *et al.*, 2009) and *Rhynocoris longifrons* (Kumar *et al.*, 2009) resulted in changes in the predatory behaviour and increase in cannibalism. In the present study, the hitherto unknown impact of intraspecific competition in the predation of cotton bollworm, *Helicoverpa armigera* (Hübner) by another harpactorine reduviid, *Scipinia horrida* (Stål) was investigated. This information is essential to avoid competition in mass rearing this reduviid for use as a biocontrol agent against the cotton bollworm and other insect pests (Ambrose, 2003). *S. horrida* also preys upon insect pests such as the mango gall midge, *Erosomyia indica*, the

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termite *Odontotermes obesus*, hairy caterpillars *Euproctis fraternal* and *E. scintillans* and the castor spiny caterpillar, *Ergolis merione*.

Adults of *S. horrida* were collected from Sunkankadai Scrub Jungle (Lat. 77° 26′E and Long. 8° 16′N) Kanyakumari district, Tamil Nadu, South India and were reared in the laboratory on *H. armigera* larvae. Two day old nymphal stages (starved for one day) and adult predators from the second generation of laboratory culture were used in the experiments. Each stage was taken in plastic containers (16 X 7 cm) at four densities, viz., 1, 2, 4 and 8 predators per container. A single second instar cotton bollworm was placed into each container. There were six life stages of the predator, each at 4 densities and thus the experiment had 24 treatments and each treatment was replicated six times. The time taken to capture, paralyze, and feed the prey and total duration of predatory events (from capturing to termination of feeding) and the number of piercing and feeding sites were recorded. The percentage increase or decrease in time taken for capturing, paralyzing and feeding, total predation and the number of piercing and feeding sites were calculated. Results were analyzed with Student's t test.

The effects of intraspecific competition in the predatory acts of *S. horrida* are presented in Table 1. Intraspecific competition significantly decreased the latency of predatory behaviour in all the life stages of *S. horrida*. For example, latency to capture, paralyze and ingest the prey was reduced by 90, 84.35 and 64.58 % when eight first nymphal instars competed for a *H. armigera* larva compared to one first nymphal instar. Similar reduction in capturing, paralyzing and sucking durations as a function of intraspecific competition has been reported for other reduviid predators such as *Catamiarus brevipennis* (Ambrose *et al.*, 1985a), *Rhynocoris marginatus* (Ambrose *et al.*, 1985b), *Acanthaspis pedestris* (Ambrose and Amudha, 1987), *R. longifrons* (Kumar *et al.*, 2009) and *I. armipes* (Das *et al.*, 2009). Ambrose and Sahayaraj (1990) reported that a peiratine reduviid *Ectomocoris tibialis*, when reared in 40 ml containers paralyzed its prey quicker than its counterparts reared in 80 and 160 ml containers. Similar situation was observed when more predators shared an arena, thereby reducing the per capita space available for each individual predator (Ambrose, 1999). In our study the per capita space, when eight *S. horrida* were enclosed together was 25 ml, compared to 200 ml when a single predator was used.

The reduction in sucking duration was more prominent in old (III to V) nymphal instars and adults than in young nymphal instars (I and II). This may be due to the congregational feeding habits of young nymphal instars that share prey whereas old nymphal instars and adults are naturally solitary predators.

Due to the reduction in capturing, paralyzing and sucking durations in *S. horrida* as a function of intraspecific competition, the total duration of predatory events significantly decreased. For example, for fifth nymphal instars of *S. horrida* with competitors the total duration of predatory events decreased by 41 % with 2 competitors and 78 % with 8 competitors. A similar reduction in the total duration of predatory acts as a function of intraspecific competition was recorded for *A. pedestris* (Ambrose and Amudha, 1987).

TABLE 1. Impact of intraspecific competition on the predatory behaviour of *Scipinia horrida* on *Helicoverpa armigera* larva

Life stage of *S. horrida*	No. of predators	Time taken for predatory acts (min) ±SD				No. of piercing and sucking sites
		Capturing	Paralyzing	Sucking	Total duration	
First nymphal instar	1	3.60 ± 0.40	10.80 ± 0.92	232.16 ± 15.10	246.56 ± 12.60	25.10 ± 1.10
	2	1.10 ± 0.09 (−69.4)	6.20 ± 0.51 (−42.59)	161.23 ± 7.20 (−30.55)	168.53 ± 8.30 (−31.64)	32.30 ± 1.60 (−11.15)
	4	0.62 ± 0.08 (−82.77)	3.40 ± 0.24 (−68.52)	96.56 ± 8.20 (−58.4)	100.58 ± 6.40 (−59.21)	18.40 ± 1.10 (−26.69)
	8	0.36 ± 0.04 (−90.0)	1.69 ± 0.18 (−84.35)	82.24 ± 5.60 (−64.58)	84.29 ± 4.60 (−65.8)	16.20 ± 1.30 (−35.46)
Second nymphal instar	1	2.90 ± 0.30	6.90 ± 0.56	184.60 ± 8.60	188.60 ± 15.60	21.20 ± 1.60
	2	0.84 ± 0.07 (−71.03)	4.30 ± 0.51 (−37.68)	106.20 ± 11.30 (−42.47)	111.34 ± 8.60 (−40.97)	18.20 ± 1.90 (−14.15)
	4	0.46 ± 0.05 (−84.14)	2.60 ± 0.03 (−62.32)	68.60 ± 6.10 (−62.84)	71.66 ± 5.10 (−62.0)	13.90 ± 0.90 (−34.43)
	8	0.24 ± 0.02 (−91.72)	1.12 ± 0.03 (−83.77)	49.30 ± 3.90 (−73.29)	50.66 ± 3.80 (−73.14)	11.80 ± 0.82 (−44.34)
Third nymphal instar	1	2.10 ± 0.20	5.80 ± 0.46	168.40 ± 10.40	176.30 ± 14.30	19.60 ± 1.20
	2	0.69 ± 0.07 (−67.14)	3.70 ± 0.20 (−36.21)	99.20 ± 7.20 (−41.09)	103.59 ± 8.20 (−41.24)	17.60 ± 1.80 (−10.2)
	4	0.38 ± 0.02 (−81.9)	2.00 ± 0.03 (−65.52)	56.70 ± 4.90 (−66.33)	59.08 ± 3.80 (−66.49)	13.20 ± 1.60 (−32.65)
	8	0.20 ± 0.01 (−90.48)	0.90 ± 0.08 (−84.48)	38.20 ± 3.10 (−77.32)	39.30 ± 1.40 (−77.7)	11.20 ± 1.40 (−42.86)

TABLE 1. (cont. . . .)

Life stage of *S. horrida*	No. of predators	Time taken for predatory acts (min) ±SD				No. of piercing and sucking sites
		Capturing	Paralyzing	Sucking	Total duration	
Fourth nymphal instar	1	1.90 ± 0.24	5.10 ± 0.56	158.20 ± 9.20	165.20 ± 9.60	17.80 ± 1.60
	2	0.59 ± 0.06 (−68.95)	3.10 ± 0.36 (−39.22)	95.60 ± 7.40 (−39.57)	99.29 ± 6.40 (−39.9)	16.90 ± 1.20 (−5.06)
	4	0.32 ± 0.04 (−83.16)	1.80 ± 0.14 (−64.71)	42.40 ± 4.60 (−73.2)	44.52 ± 3.60 (−73.05)	12.20 ± 1.60 (−31.46)
	8	0.16 ± 0.01 (−91.58)	0.90 ± 0.06 (−82.35)	31.20 ± 2.90 (−80.28)	32.26 ± 3.80 (−80.47)	10.80 ± 1.10 (−39.33)
Fifth nymphal instar	1	1.60 ± 0.18	4.20 ± 0.34	128.40 ± 9.40	134.20 ± 11.60	15.40 ± 1.20
	2	0.50 ± 0.04 (−68.75)	2.60 ± 0.24 (−38.1)	76.30 ± 6.40 (−40.58)	79.40 ± 6.20 (−40.83)	12.10 ± 1.10 (−21.43)
	4	0.24 ± 0.01 (−85.0)	1.10 ± 0.14 (−73.81)	31.80 ± 4.20 (−75.23)	33.14 ± 2.60 (−75.31)	11.40 ± 1.20 (−25.97)
	8	0.11 ± 0.02 (−93.13)	0.80 ± 0.07 (−80.95)	28.60 ± 2.40 (−77.73)	29.51 ± 1.80 (−78.01)	10.10 ± 0.94 (−34.42)
Adult	1	1.10 ± 0.18	3.60 ± 0.52	102.40 ± 8.16	107.10 ± 8.80	15.20 ± 1.20
	2	0.41 ± 0.06 (−62.73)	2.10 ± 0.30 (−41.66)	94.20 ± 6.40 (−8.01)	96.71 ± 7.40 (−9.7)	11.20 ± 1.40 (−26.32)
	4	0.17 ± 0.02 (−84.55)	0.96 ± 0.07 (−73.33)	51.60 ± 4.40 (−49.6)	52.73 ± 5.60 (−50.77)	10.60 ± 0.90 (−30.26)
	8	0.09 ± 0.008 (−91.82)	0.72 ± 0.06 (−80.0)	44.4 ± 2.80 (−56.64)	45.21 ± 3.20 (−57.79)	9.60 ± 0.84 (−36.84)

Values given are mean of 6 observations. Values in parentheses indicate percentage of increase or decrease over corresponding control values. All deviations are significant at $P \leq 0.05$.

The reduction in number of piercing and sucking sites as a function of intraspecific competition in proportion to the number of competitors could be attributed to the lesser number of sites available for competitors due to congregational feeding. However, solitary adult predators also pierced and sucked at lesser number of sites, perhaps due to the constant threat they perceived from the competitors. In *E. tibialis* decrease in the number of piercing and sucking sites resulted as a function of a reduction in rearing space (Ambrose and Sahayaraj, 1990).

The observations presented here on the impact of intraspecific competition on predatory events by *S. horrida* revealed the predatory patterns in congregational young nymphal instars and solitary old nymphal instars and adults of *S. horrida*. The impact of intraspecific competition is greater in older nymphs. This understanding on the effects of intraspecific competition on predation will help to determine the optimum number of life stages of *S. horrida* to be used for mass rearing of the predator for biological control programmes.

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Impact of Synergy-505 (chlorpyriphos 50% + cypermethrin 5%) on the biology and life table parameters of the assassin bug, *Rhynocoris marginatus* (Fabricius) (Hemiptera: Reduviidae)

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ABSTRACT: The effects of exposure of sublethal concentrations of Synergy-505 (chlorpyriphos 50% + cypermethrin 5%) on the biology and life table parameters of a nontarget biological control agent, *Rhynocoris marginatus* (Fabricius) were studied in the laboratory. In Synergy-505 exposed *R. marginatus*, the stadial period and incubation period increased over control. Adult longevity, ovipositing days and fecundity had marked reduction while stadial period and pre oviposition days got slightly prolonged. In life table parameters, gross and net reproductive rates and hypothetical population in F2 generation registered heavy reduction. Other parameters also showed lower levels but differences were not marked.

KEYWORDS: *Rhynocoris marginatus*, Synergy-505, insecticide, biology, life table

The assassin bug, *Rhynocoris marginatus* (Fabricius) (Hemiptera: Reduviidae) is an important predator of insect pests such as *Helicoverpa armigera* (Hübner) and *Spodoptera litura* (Fabricius) in agroecosystems in south India (Ambrose, 1999). Insecticide combinations are being used by cultivators for controlling paddy pests. Synergy-505 (chlorpyriphos 50% + cypermethrin 5%) is one such combination used in paddy fields extensively. Sublethal doses of cypermethrin (one component of Synergy-505) were reported to show adverse effect on population growth parameters of *R. marginatus*. But information on the impact of Synergy-505 is lacking and hence, the present study was aimed to understand its impact on the biology and life table parameters of *R. marginatus*.

Third nymphal instars, 0-day old, of *R. marginatus* collected from a laboratory culture were continuously exposed to sublethal concentrations of Synergy-505 for 20

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TABLE 1. Effect of sublethal concentration of Synergy-505 on the biological parameters of *Rhynocoris marginatus*

Parameter	Control	Synergy-505
Incubation period (d)	8.26	9.21
Stadial period (d)		
III instar	8.50[a]	12.34[b]
IV instar	11.14[c]	14.80[d]
V instar male	13.83[e]	18.20[f]
V instar female	16.14[g]	22.00[h]
Nymphal mortality (%)	0	23
Adult longevity (d)		
Male	79.00	59.83
Female	97.71	63.00
Pre-oviposition period (d)	14.00	16.75
No. of ovipositing days	68.39	34.78
Total number of eggs laid	230.86	113.57
Total number of nymphs hatched	210.43	106.00
Hatching percentage	97.27	93.72
Total longevity (d)		
Male	138.17	125.80
Female	159.14	137.00
Sex ratio (male: female)	1:1.16	1:1.28

The values superscribed with different letters are statistically significant at $P \leq 0.05$.

days by enclosing them inside petridishes containing insecticide applied absorbent paper. The treated paper was replaced daily. Bioassay studies were carried out following the procedure of Sprague (1973) and from the mortality data for 48 h of exposure, LC_{50} value of Synergy-505 i.e., 4 μl, was computed following Finney (1971). One tenth of this dose i.e., 0.4 μl, was selected as sublethal dose for the experiments. The data on the life table parameters viz. incubation and stadial periods, fecundity and longevity were recorded. Data were subjected to DMRT for comparisons of means. The life table was constructed according to Southwood (1978) and Bellows *et al.* (1992).

The results presented in Table 1 show that the sublethal dose of synergy-505 significantly increased the duration of 1st 2nd and 3rd nymphal stadia to about 12, 15 and 18 days compared to 8, 11 and 13 days in corresponding controls. Similarly, the preoviposition period (from 14 d in control to 17 d in treatment) and incubation period (8 to 9 d) also showed an increasing trend in treatment.

The adult longevity in treated insects were much lower (male 60 d, female 63 d) compared to those in control (79 d and 98 d, respectively). There was drastic reduction in the number of eggs laid by treated insects (113/female) and number of hatching eggs (106) compared to those in control (231 and 210, respectively). But the fall in hatching

TABLE 2. Life table parameters of *Rhynocoris marginatus* exposed to Synergy-505 in comparison with control

Parameter	Control	Synergy-505
Gross reproductive rate (Σm_x)	273.62	129.46
Net reproductive rate ($R_o = \Sigma l_x m_x$)	230.83	88.02
Mean length of generation ($T_c = \Sigma l_x m_x x / R_o$)	109.12	103.39
Estimated value of intrinsic rate of increase in numbers (r_c)	0.050	0.043
Corrected r_m ($e^{7-rmx} l_x m_x = 1096.6$) (female/female/day)	0.0635	0.0530
True generation time ($T = \log_e R_o / r_m$)	85.669	84.48
Finite rate of increase in numbers ($\lambda = \text{antilog}_e r_m$)	1.066	1.054
Doubling time (days) ($\log 2 / \log \lambda$)	10.86	13.20
Weekly multiplication rate (WMR= antilog (e^{rm})^7)	1.559	1.449
The adult attained mean progeny production (female/day)	2.880	3.319
Hypothetical population in F2 generation = $(R_o)^2$	53282.48	7747.52
Annual rate of increase	1.16×10^{10}	2.5×10^8

percentage was not high (from 97 to 94 only). The prolongation of the pre-oviposition period and the development period and the reduction of fecundity were reported in *R. marginatus* exposed to sublethal doses of synthetic pyrethroids like deltamethrin and cypermethrin (Ambrose, 1999; Ambrose *et al.*, 2006, 2007).

Synergy-505 showed significant toxicity to the progeny as shown by 23% mortality in larval stages as against no mortality in control (Table 2). The adverse effect of Synergy-505 appears to be largely on egg production and to a limited extent on prolongation of developmental duration. Though synthetic pyrethroids are claimed to be comparatively safer to natural enemies when used in field, the present findings indicate a possible adverse effect on progeny production. Ambrose *et al.* (2006, 2007)) also observed similar effect of deltamethrin and cypermethrin. Dhingra (1999) reported that among various pyrethroids, cypermethrin was the most toxic to the predatory bugs. Though the exact mechanism of the above effects of sublethal doses of insecticides is not fully understood, Conney *et al.* (1967) suggested that the insecticide residues may reduce the availability of ecdysone and the view was supported by Ambrose (1999).

The life table (Table 2) also revealed significant variations between control and Synergy-505 exposed *R. marginatus*. In exposed insects there was gross fall in reproductive rate (130 in treatment and 273 in control), net reproductive rate (88 and 231) and hypothetical population in F_2 generation (7742 and 53282). In the remaining parameters of the life table also, the values were lower in treatment compared to control, except in doubling time where the value was higher (13 d) compared to control (10 d). The results clearly show that the application of Synergy-505 in the agroecosystem will reduce the build up of the field population of *R. marginatus*.

The normal innate capacity of increase of 0.05 per female per day was reduced to 0.043 in Synergy-505 treated *R. marginatus*. Such a reduction in innate capacity of increase could be correlated to the reduced fecundity and hatchability observed in

insecticide exposed predators. Though Synergy-505 did not alter the daily infinite rate of increase, the population doubling time was increased from 10.86 d in control to 13.2 days. Synergy-505 also did not affect the weekly multiplication rate (Table 2). The sublethal doses of Synergy-505 altered the parameters of population growth and thereby reduced the hypothetical females in F_2 generations from 53282.48 in control to 7747.52 (Table 2). Similar observations were made by Ambrose *et al.* (2006, 2007)) in deltamethrin and cypermethrin exposed *R. marginatus*. The variations in the life table parameters of *R. marginatus* treated with Synergy-505 clearly revealed that it reduced the reproductive capacity and longevity and prolonged the developmental time. These studies suggest that the use of Synergy-505 against insect pests in agroecosystems wherein *R. marginatus* inhabits could affect the physiology of this non-target beneficial.

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Rapid differentiation of the sexes in adult *Sitophilus oryzae* (L.) (Coleoptera: Rhynchophoridae)

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ABSTRACT: Sexual dimorphism in external morphological characters of the common rice weevil, *Sitophilus oryzae* (L.) was studied. Nature and morphometrics of rostrum, prothorax and elytra were found reliable for easy and rapid differentiation of the sexes in *S. oryzae* on the basis of genitalial dissections and SEM studies.

KEYWORDS: *Sitophilus oryzae*, sexual dimorphism, genitalia, morphometrics

The results revealed that the adults can be sexed by the dimensions of the rostrum and the punctations. Compared to females, males have a short, thick-stout rostrum, which is straight, much wider at the middle than at apex and base; lateral and dorsal surface with very coarse irregular punctations giving a rough appearance; distance between apex of rostrum and insertion of scape on the scrobe 0.88x shorter than in female; and elytra and prothorax 0.94x and 0.92x shorter than in females (Table 1, Figs. 3a–c & 4a–c).

Among stored products pests, the genus *Sitophilus* contains three of the world's most destructive pests, *Sitophilus oryzae* (L.), *S. zeamais* Motschulsky and *S. granarius* (L.). Among these, the common rice weevil, *S. oryzae* is a cosmopolitan pest causing immense economic damage. The three species have great morphological resemblance and for identifying them to species level the separation of the sexes is a pre-requisite. It also becomes essential in go-downs while assessing infested commodities for quarantine purpose. It is desirable to identify easily recognizable morphological differences similar to those available for other weevil species like *Calandra granaria, S. zeamais* and *S. granarius* ((Andersen, 1938); El-Sayed and Rostom, 1962; Procter, 1971). Keeping this in mind, sexual dimorphism in various external morphological features of *S. oryzae* was explored.

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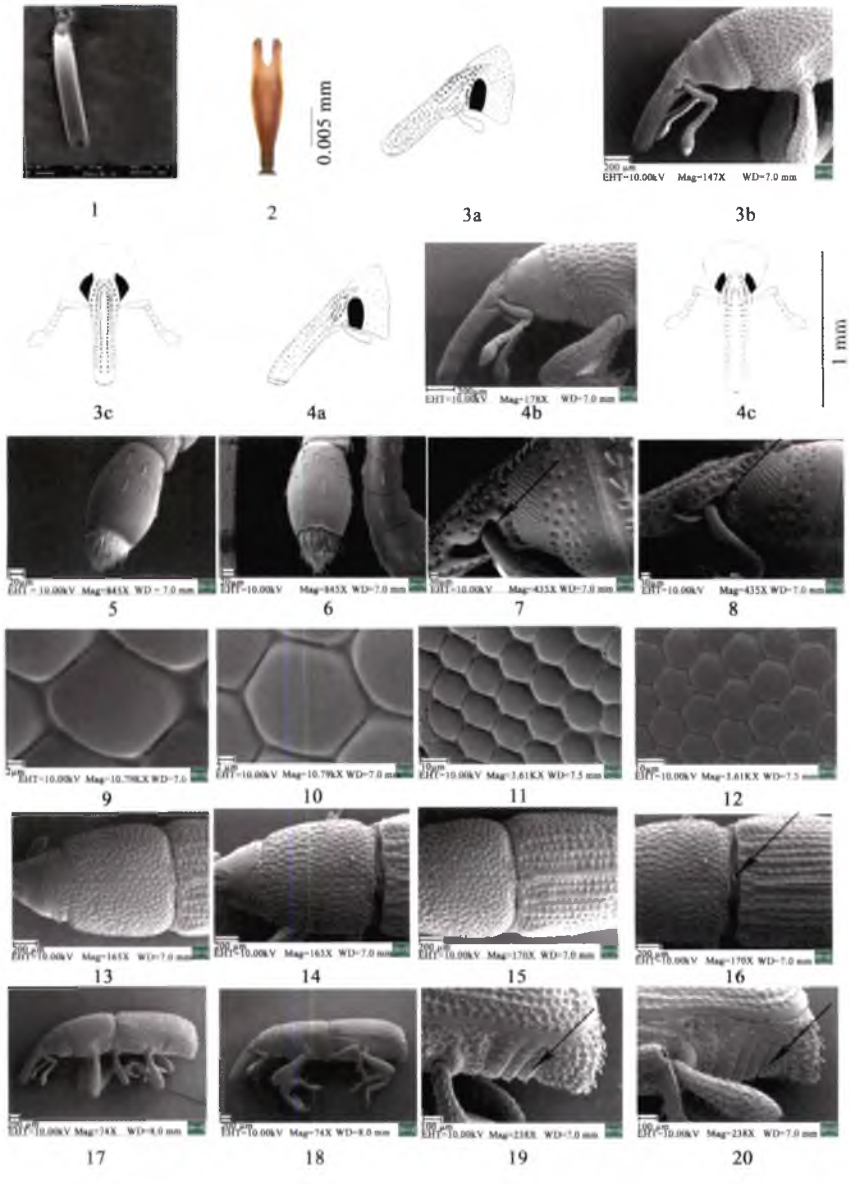

FIGURES 1–20. *Sitophilus oryzae* (L.): 1–2, genitalia: 1, aedeagus (dorsal view); 2, female sclerite (dorsal view); 3, Rostrum of male: a–b, lateral view; c, dorsal view; 4, Rostrum of female: a–b, lateral view; c, dorsal view; 5, antennal club male; 6, antennal club female; 7, scrobe male; 8, scobe female; 9, individual ommatidia male; 10, individual ommatidia female; 11, ommatidia per unit area male; 12, ommatidia per unit area female; 13, puctations on prothorax male; 14, punctations on prothorax female; 15, visible mesonotum male; 16, visible mesonotum female; 17–18, male with flattened abdomen; 19–20, female with curved abdomen.

TABLE 1. Sexual dimorphism in adult *Sitophilus oryzae*

	Measurements (mean ± SE, in mm) of								
	Rostrum							Prothorax	Elytra
Sex	Length (dorsal view)	Length (lateral view)	Length (ventral view)	Distance between posterior margin and insertion of scape	Width at apex (dorsal view)	Width at middle (dorsal view)	Width at base (dorsal view)	Length (dorsal view)	Length (dorsal view)
Male	0.37 ± 0.004	0.34 ± 0.004	0.29 ± 0.005	0.30 ± 0.004	0.12 ± 0.001	0.08 ± 0.0009	0.089 ± 0.0008	0.50 ± 0.008	0.65 ± 0.001
Female	0.41 ± 0.007	0.38 ± 0.68	0.39 ± 0.68	0.34 ± 0.005	0.13 ± 0.001	0.07 ± 0.0008	0.097 ± 0.001	0.54 ± 0.006	0.69 ± 0.007
t value	3.90**	4.88**	12.30**	5.19**	2.19*	7.99**	8.33**	2.90*	4.07**
F value	15.20**	27.58**	150.84**	26.89**	4.78*	81.00**	69.41**	14.91**	16.56**

** Significant at 1 %; *Significant at 5%

Fourteen days old adults of *S. oryzae* were randomly chosen from a population maintained in the laboratory at 25° ± 2°C and 30 ± 5% RH, and 30 numbers each of males and females were observed under Leica EZ4 stereozoom microscope. The sex of the selected adults was confirmed through genitalial dissection (Fig. 1, 2). Data on the morphometrics of each individual *viz.*, length of rostrum (lateral, dorsal and ventral), width of rostrum at middle, anterior and posterior margin, distance between insertion of scape on the scrobe and anterior margin of rostrum, and length of prothorax and elytra (greatest distance between apex and base) were measured under stereozoom microscope and illustrations prepared with a drawing tube. Photographs were made with Leica DFC290 photoautomat. Surface morphology was studied under high vacuum SEM with ZEISS EVO MA 10 at 10 Kv after palladium coating.

The SEM observations also revealed that there are other significant differences in the two sexes: Compared to females, males have their antennal club with short sensillae (Fig. 5 & 6), inner margin of scrobe more convex, deep, posteriorly wide (Fig. 7 & 8), ommatidia not wholly hexagonal, and more per unit area (Fig. 9, 10 & 11, 12); punctations on the pronotum deep (Fig. 13 & 14); base of pronotum and elytra separated by a short belt with "visible part of mesonotum" smaller (Fig. 15 & 16); and abdomen not curved at apex (Fig. 17, 18 & 19, 20).

Statistical analysis of the data [students t test and single factor ANOVA ($n = 30$)] on morphometrics of antennae, rostrum, prothorax and elytra revealed that the variations between males and females were significant (Table 1).

The morphometrics of rostrum, prothorax and elytra, and the punctations on the rostrum will aid in quick and reliable separation of sexes in *S. oryzae*. The separation of sexes done using the above characters agreed with the results of genitalial dissections. Some of the characters, especially insertion of scape on the scrobe on the rostrum, thickness of rostrum and nature of punctations have been utilized for differentiation of sexes in other weevils (Halstead, 1964; Proctor, 1971; Dobie *et al.*, 1991), thus corroborating the conclusions of the present investigations.

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